Official Organ of the Society for the Study of Development and Growth

GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

UNIVERSITY
OF MICHIGAN
SEP 6 1951
PERIODICAL
READING ROOM

VOLUME XV, NUMBER 2
PAPERS 392-396

JUNE, 1951 **$6.00 A YEAR**

Entered as second class matter on October 20, 1941, at the Post Office, Worcester, Mass., under the Act of March 3, 1879.

GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy, and anthropology; just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamins, and hormones.

Published four times a year during March, June, September and December. Subscription beginning with the first issue of Volume XV is $6.00 per volume (outside U. S. and Canada, $6.50). Single numbers $2.00. There will be one volume of at least 400 pages each year.

All manuscripts and subscriptions should be sent to: The Editors of Growth, Lankenau Hospital Research Institute, Fox Chase, Philadelphia 11, Pa.

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Growth, 1951, **15**, 57-78.

GENETIC DIFFERENCES IN QUANTITATIVE HISTOLOGY OF THE ADRENAL, ORGAN WEIGHTS, AND INTER-ORGAN CORRELATIONS IN WHITE LEGHORN CHICKENS[1]

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(Received for publication June 19, 1951)

Interrelationships of body and organ weights in 10 inbred lines of White Leghorn chickens and a comparison of organ weights with incidence of lymphomatosis were reported in a recent paper by Oakberg and Lucas (1949). Growth factors were divided into those which affect the organism as a whole, those specific for the liver and pancreas, and those specific for the spleen. Common factors were indicated for large liver and pancreas and susceptibility to lymphomatosis. The importance of genetic differences was demonstrated for all variables. The data presented in this paper have been extended to include thymus, pituitary, and adrenal weights, together with relative proportions of adrenal medulla and cortex in the hope of further elucidating the normal growth relationships of the organs of fowl, particularly in reference to the influence of genetic constitution.

The literature concerning growth of the total body and of individual organs in various mammals and birds has been reviewed previously (Oakberg and Lucas, 1949). Accordingly, attention will be directed primarily to a consideration of pituitary and adrenal weights, and their relation to growth and regression of the thymus and other lymphoid tissues.

Variation in weight of the pituitary probably arises in part from

[1]This work was supported by a grant made by Swift and Company, Chicago, Illinois, to the Agricultural Experiment Station of Michigan State College. Laboratory facilities, equipment, and material were furnished by the U. S. Regional Poultry Research Laboratory.

Journal Article No. *1231* of the Michigan Agricultural Experiment Station, East Lansing, Michigan.

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errors in dissecting and weighing such a small gland. However, ability to demonstrate significance of pituitary weight differences in different groups of animals demonstrates clearly that some of the variations observed are real. Certainly this variation is widespread as is indicated by the summary of literature in Table 1. Likewise, variation in adrenal weights is considerable, but larger size of the glands facilitates comparisons and establishment of differences. Some of the more pertinent literature on adrenal weights also is summarized in Table 1.

That species characteristics exist is evident; also, discrepancies in results of investigations on the same species may depend upon genetic differences in the stocks used. Breeding for specific genes, together with selection for tameness, as suggested by Keeler (1947), probably is a large factor in determining the smaller relative adrenal size in domesticated versus wild species (Hatai, 1914; Rogers and Richter, 1948; Quiring, 1938). Rogers and Richter (1948) demonstrated that smaller size of the adrenal in tame Norway rats was due to a reduction in size of the cortex.

Growth and regression of the thymus has been the subject of voluminous literature which has been reviewed by Boyd (1932) and Hammar (1921a, 1921b). Without exception, thymus weight has been observed to be extremely variable. Recently the elucidation of the alarm reaction (Selye, 1936, 1937) and the general adaptation syndrome (Selye, 1946) has greatly clarified the behavior of the thymus in health and disease. It is not within the scope of this paper to review the extensive literature which has established the importance of the hormones in development and function of the lymphoid, reticuloendothelial, and hemopoietic systems. This subject has been treated by Dougherty and White (1944, 1945, 1947); Jones (1949); Kendall (1941); Li and Evans (1947); Reichstein and Shoppe (1943); Reiss (1947); Sarason (1943); Selye (1936, 1937, 1946); Skelton (1950); Swann (1940); Tepperman, et al. (1943); Valentine, et al. (1948); Vogt (1947); and White (1949). Sayers and Sayers (1947) summarize the physiological relationship as a production of ACTH by the pituitary in response to various stresses, with subsequent stimulation of the adrenal cortex to secrete the 11-oxygenated steroid hormones which cause involution of the thymus and lymphoid tissues. However, Fortier et al. (1950) have indicated that there are peripheral factors independent of the pituitary which maintain the discharge of adrenal cortical hormone after the initial alarm reaction. There appears to be no question that the pituitary, through secretion of adreno-corticotropic

TABLE 1

SUMMARY OF LITERATURE ON PITUITARY AND ADRENAL WEIGHTS

Gland studied	Experimental animal	High variation in gland weight observed	No sex differences	Gland relatively heavier in males	Gland relatively heavier in females	Genetic differences observed
Adrenal	Guinea Pig	Bessesen and Carlson, 1923 Eaton, 1938	Bessesen and Carlson, 1923 Eaton, 1938 Mixner et al., 1943			Strandskov, 1937
	Rat	Jackson, 1913 Freudenberger, 1932 Rogers and Richter, 1948 Addis and Gray, 1950	Rogers and Richter, 1948 Addis and Gray, 1950		Hatai, 1913, 1914 Jackson, 1913 King and Donaldson, 1929 Donaldson, 1924	Keeler, 1947 King and Donaldson, 1929 Yeakel and Rhoades, 1941
	Rabbit		Allanson, 1932		Kibler et al., 1942	Robb, 1929
	Cat	Latimer, 1939				
	Mouse					Stein and Cheng, 1948
	Chicken	Juhn and Mitchell, 1929 Landauer and Aberle, 1935 Breneman, 1941	Juhn and Mitchell, 1929 Landauer and Aberle, 1935			Landauer and Aberle, 1935 Leonard and Righter, 1936 Breneman, 1941
	Turkey	Latimer and Rosenbaum, 1926				
	Eng. sparrow	Quiring and Bade, 1943				
	Pigeon		McCarrison, 1919			
Pituitary	Guinea pig	Bessesen and Carlson, 1923 Eaton, 1938	Bessesen and Carlson, 1923 Mixner et al., 1943			Eaton, 1938

TABLE 1 (*continued*)

Gland studied	Experimental animal	High variation in gland weight observed	No sex differences	Gland relatively heavier in males	Gland relatively heavier in females	Genetic differences observed
	Rat	Freudenberger, 1933			Hatai, 1913, 1914 Donaldson, 1924 King and Donaldson, 1929	Keeler, 1947
	Rabbit		Livingston, 1916 Allanson, 1932			
	Cat	Latimer, 1939				
	Dog				Latimer, 1941	
	Mouse					Smith and McDowell, 1930
	Chicken	Latimer, 1924 Juhn and Mitchell, 1929	Juhn and Mitchell, 1929	Landauer and Aberle, 1935		Leonard and Righter, 1936
	Pigeon				McCarrison, 1919	Riddle, 1947
	Doves					Riddle, 1947

hormone with subsequent mediation of the adrenal cortex is of great importance, not only in periods of stress, but also in normal maintenance and function of the lymphoid and closely related tissues. This regulatory function has been demonstrated for the reticuloendothelial system (Gordon and Katsh, 1949).

With the exception of Riddle and his group (Riddle, 1947); and Keeler (1947), the importance of genetic differences in interrelationships of the endocrine system has received but scattered attention. Bates, et al., (1941), observed a marked difference in the response of different strains of White Leghorn chicks to thyrotropin, and Munro, et al., (1943) have reported breed differences in response of chickens to thyrotropin and gonadotropin. Gardner, et al. (1944) reported strain differences in the effect of various steroid hormones on leukemia in mice. Smith and MacDowell (1930) demonstrated that a single recessive gene markedly influenced the anatomy and thereby the function of the mouse pituitary. That other genes less drastic in their effects also affect the endocrine system has been shown by Keeler (1947), Riddle (1947), and Stein and Cheng (1948). Also, Brody and Kibler (1941) after an extensive survey of organ: body-weight relationship in several mammalian and avian species, concluded that the neuro-endocrine system was the limiting factor in conditioning basal metabolism.

In birds, cortical and medullary portions of the adrenal are intermingled. The histological appearance has been described by Rabl (1891), Günther (1906), Müller (1929), Sauer and Latimer (1931), and Kar (1947). It is at present impossible to segregate the cortex into cells or groups of cells corresponding to the cortical zones of mammals. That similarities exist is highly probable in view of the observation of Müller (1929) that cortical cells in the adrenals of birds pass through a cycle of growth and senescence.

Weight of organs and quantitative measurements of histological preparations cannot be considered reliable estimates of physiological activity. Hypertrophy of a gland may indicate either hyper- or hypofunction. Also, as emphasized by Gardner (1948) consideration of endocrine actions should include rate of production, rate of destruction, capacity of tissues to respond, and quality of hormone produced. If Vogt (1947) is correct in concluding that hormones are released almost immediately after secretion, biological assay and cytochemical methods also must be considered with caution. In spite of the above inadequacies, it was felt that a study of the organ-body weight and inter-

organ relationships of inbred lines of White Leghorn chickens should indicate some of the interrelationships in development of organs and tissues and the extent to which they can be altered by genetic constitution.

Materials and Methods

One hundred fifty eight, 120-day old, chickens equally divided as to sex were used. Four inbred lines from the "J" population of the Laboratory breeding flock, and four families from each line (family refers to the progeny of a single sire and dam) were represented. Forty birds were available in lines 6 and 10, and 39 birds were used in lines 11 and 15. General breeding and management procedures used have been given by Waters (1945). Lines 6 and 10 have been selected for resistance and lines 11 and 15 have been selected for susceptibility to lymphomatosis.

At 120 days after hatching the birds were weighed, killed by electrocution, and the liver, pancreas, spleen, thymus, adrenals and pituitary were removed and weighed as quickly as possible. Liver was weighed to the nearest gram; pancreas, spleen and thymus were weighed on a torsion balance sensitive to 0.2 gm.; the adrenals and pituitary were weighed on a Roller-Smith micro torsion balance.

Liver, pancreas and adrenals were fixed in Zenker formol for histological study, and the adrenals also were chromated for one week in 3 percent dichromate. Tissues were imbedded in paraffin, sectioned at 5 μ and stained with hematoxylin azure II-eosin.

A preliminary study indicated that orientation of the adrenal had no effect on the quantitative determination of amount of cortex and medulla as both types of tissue were evenly distributed throughout the gland. Accordingly, longitudinal sections were taken in order to get a larger section area, with no regard to orientation. Five sections, spaced a minimum of 100 μ apart, were taken from each gland. Relative proportions of cortex and medulla were determined by the method of Chalkley (1943). A check with planimeter measurements of tracings made with the aid of a microprojector indicated that the two methods agreed within 5 percent. Most of the difference was due to poor resolving power obtained with the microprojector, and the resulting difficulty in excluding blood vessels and connective tissues. Weights of the cortex and of the medulla were obtained by multiplication of total gland weight by percentage of the respective tissues. This gives somewhat of an overestimate of the actual value, for amount

of connective tissue, both in the capsule and adhering to the capsule, was not considered. However, as the procedure was uniform for all birds, this error should not affect the comparisons made here.

Results

Quantitative Histology of the Adrenal

Mean percentage of cortical and medullary tissue in the adrenals is given by sex, family, and line in Table 2. The total 10 sections per bird, 5 from the left and 5 from the right adrenals were combined, since variance analysis (Table 3) indicated no difference between the two sides. Family numbers given in Table 2 are arbitrary and bear no relation to pedigree records.

Considerable variation in percentages of cortical and medullary tissue occur between individuals of the same family, sex, and line. This difference is substantiated by the variance analyses given in Table 3, where highly significant mean squares are observed for percentage of cortex between birds within family and sex for lines 6, 10, and 11, and significant differences are observed in line 15. Medulla percentages are significant at the one percent level for lines 6 and 11, and at the 5 percent level for lines 10 and 15. In all lines, highly significant differences between families were observed both for cortex and medulla. Differences between lines were highly significant for both cortex and medulla. The sex comparison is an interesting one with no significance in line 6, highly significant differences both for cortex and medulla in line 10, significance for cortex in line 11, and for medulla in line 15. Thus all possible relationships of sex differences in percentage of cortex and medulla are represented by the four lines. When based on all birds, none of the lines shows a significant difference in percentage composition of the right and left sides. However, the bird x side interaction is highly significant in each line. Accordingly, the common practice of using one adrenal as a control (as in a check of chemical analysis with histological preparations), must be based on a large enough sample to balance differences which occur in single individuals.

Body and Organ Weights

Gross weight.—Mean body and organ weights with standard deviations, and organ:body weight ratios are given by sex and line in Table 4. Variance analysis of the gross weight indicated that the body and organs of the males were significantly heavier than those of the

TABLE 2

FAMILY, SEX, AND LINE MEAN PERCENTAGE OF CORTICAL AND MEDULLARY TISSUE IN THE ADRENALS OF 120-DAY OLD WHITE LEGHORN CHICKENS FROM FOUR INBRED LINES

	Line 6				Line 10				Line 11				Line 15			
	Males		Females		Males		Females		Males		Females		Males		Females	
	medulla	cortex	medulla	cortex	medulla	cortex	medulla	cortex	medulla	cortex	medulla	cortex	medulla	cortex	medulla	cortex
Family 1	37*	58*	35	60	45	50	39	57	48	48	48	48	51	45	48	48
Family means			36	59			42	54			48	48			49	46
Family 2	38	57	42	53	45	51	38	57	52	42	47	48	53	44	50	46
Family means			40	55			41	54			50	45			51	45
Family 3	41	55	42	53	46	50	41	55	53	42	49	46	47	50	45	51
Family means			42	54			43	52			51	44			46	50
Family 4	37	59	34	61	40	56	36	60	46**	49**	46	49	52	43	53**	41**
Family means			36	60			38	58			46	49			53	43
Sex x line means	38	57	39	57	44	52	38	57	50	45	48	48	51	45	49	47
Line means			38	57			41	54			49	46			50	46
Sex means for entire experiment													46	50	43	52

* Mean of 5 birds
** 4 Birds for these means

TABLE 3
VARIANCE ANALYSES, BY LINES, OF PERCENTAGES OF CORTEX AND MEDULLA

Source of Variation	Degrees of freedom	Mean squares			
		Line 6		Line 10	
		Medulla	Cortex	Medulla	Cortex
Total	399				
Sex	1	2.25	15.68	2,984.93**	3,106.61**
Side	1	3.12	5.93	2.24	26.38
Families	3	898.04**	995.54**	554.23**	524.14**
Sex x side	1	16.83	19.07	9.63	61.89
Sex x families	3	220.62	219.19	29.08	29.63
Side x families	3	43.54	29.05	84.25	78.37
Sex x side x families	3	66.97	36.63	31.06	21.34
Birds within family and sex	32	115.14**	121.56**	76.66*	98.45**
Birds within family and sex x side	32	39.14**	48.52**	36.96**	31.00**
Within birds, family, sex side	320	16.40	15.42	15.43	15.26
Pooled error:					
For testing sex	7	125.66	112.36	27.15	30.69
For testing families	9	110.38	94.96	48.13	43.11
		Line 11		Line 15	
Total	389				
Sex	1	440.26	686.07*	393.26*	176.97
Side	1	160.25	228.71	148.51	81.81
Families	3	453.25**	582.80**	853.59**	928.75**
Sex x side	1	201.67	273.21	43.03	4.24
Sex x families	3	143.66	137.69	72.32	91.47
Side x families	3	15.60	20.21	118.99	164.02
Sex x side x families	3	29.66	32.67	49.52	60.55
Birds within family and sex	31	230.01**	210.24**	96.64*	102.70*
Birds within family and sex x side	31	37.24**	42.43**	47.49**	49.90**
Within birds, family, sex and side	312	15.95	15.67	18.16	18.72
Pooled error:					
For testing sex	7	103.09	112.04	58.36	65.76
For testing families	9	62.97	63.52	80.28	105.35

* Significant at 5% level
** " " 1% "

females, with the exception of the thymus, where no significant differences were observed. As these differences probably are influenced by the heavier body weight of the males, analysis by the covariance procedure (as given by Snedecor, 1946) was used in determining significant differences for sex, lines and families within lines (Table 5).

Correction of organ weights for body weight removed the differences between the sexes for all organs except the total adrenal, adrenal cortex, adrenal medulla, and pituitary. The significance of the total adrenal is due to a relatively heavier gland in the males of lines 6,

TABLE 4

MEAN BODY AND ORGAN WEIGHTS, IN GRAMS, AND ORGAN:BODY-WEIGHT RATIOS FOR 120-DAY OLD CHICKENS FROM 4 INBRED LINES

	Line 6				Line 10			
	20 Males		20 Females		20 Females		20 Females	
No. of individuals	Mean wt.	Organ wt. / Body wt. × 100	Mean wt.	Organ wt. / Body wt. × 100	Mean wt.	Organ wt. / Body wt. × 100	Mean wt.	Organ wt. / Body wt. × 100
Body wt.	1,540 ± 181*		1,179±122		1,447 ± 131		1,138 ± 67	
Liver	40.2 ±6.00	2.63	28.5 ±3.55	2.43	31.5 ±3.38	2.19	26.0 ±5.74	2.28
Pancreas	2.78 ±0.42	0.18	2.41 ±0.38	0.21	2.99 ±0.40	0.21	2.61 ±0.25	0.23
Spleen	2.66 ±0.43	0.17	2.32 ±0.46	0.20	3.19 ±0.73	0.22	2.84 ±0.34	0.25
Thymus	4.19 ±1.05	0.27	4.23 ±0.78	0.36	5.26 ±1.38	0.37	5.82 ±1.73	0.51
Adrenal	0.182±0.025	0.012	0.134±0.019	0.011	0.154±0.015	0.011	0.115±0.011	0.010
Adrenal cortex	0.104±0.015	0.007	0.076±0.011	0.006	0.079±0.009	0.005	0.066±0.009	0.006
Adrenal medulla	0.070±0.012	0.005	0.052±0.011	0.004	0.067±0.009	0.005	0.044±0.005	0.004
Pituitary	0.011±0.0017	0.0007	0.008±0.0018	0.0004	0.010±0.0015	0.0007	0.007±0.0012	0.0006

	Line 11				Line 15			
No. of individuals	19 Males		20 Females		20 Males		19 Females	
Body wt.	1,774 ± 180		1,268 ± 98		1,703 ± 123		1,197 ± 55	
Liver	38.8 ±6.16	2.19	26.7 ±2.62	2.11	34.4 ±4.59	2.02	22.5 ±2.32	1.88
Pancreas	3.74 ±0.54	0.21	2.95 ±0.48	0.23	2.81 ±0.33	0.17	2.45 ±0.28	0.20
Spleen	3.56 ±0.64	0.20	2.83 ±0.58	0.22	2.71 ±0.41	0.16	2.35 ±0.32	0.20
Thymus	4.17 ±1.08	0.23	4.70 ±1.55	0.37	5.23 ±1.40	0.31	5.86 ±1.21	0.49
Adrenal	0.158±0.022	0.009	0.116±0.017	0.009	0.187±0.015	0.011	0.122±0.013	0.010
Adrenal cortex	0.071±0.011	0.004	0.055±0.008	0.004	0.085±0.008	0.005	0.057±0.009	0.005
Adrenal medulla	0.079±0.015	0.004	0.055±0.010	0.004	0.095±0.013	0.006	0.059±0.008	0.005
Pituitary	0.009±0.0015	0.0005	0.008±0.0018	0.0006	0.011±0.0027	0.0007	0.009±0.0019	0.0008

* Standard deviation

TABLE 5

SIGNIFICANCE OF ORGAN WEIGHT DIFFERENCES BETWEEN SEX, LINES AND FAMILIES WITHIN LINES AFTER CORRECTION FOR BODY WEIGHT BY COVARIANCE

Source of Variation	Degrees of freedom	Liver wt.	Pancreas wt.	Spleen wt	Thymus wt.	Adrenal wt.	Adrenal cortex wt.	Adrenal medulla wt.	Pituitary wt.
Sex	1	N.S.	N.S.	N.S.	N.S.	0.01	0.05	0.05	0.01
Lines	3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Families within lines	12	N.S.	N.S.	N.S.	N.S.	0.01	0.01	0.05	N.S.

N.S. — No significance
0.05 — F value exceeds 5% level of significance
0.01 — F value exceeds 1% level of significance

TABLE 6

SIGNIFICANCE OF VARIATION BETWEEN FAMILIES WITHIN LINES IN RESPECT TO WEIGHT OF THE TOTAL ADRENALS, ADRENAL CORTEX, AND ADRENAL MEDULLA AFTER CORRECTION FOR BODY WEIGHT BY COVARIANCE

Line	Total adrenal wt.	Wt. of adrenal cortex	Wt. of adrenal medulla
6	0.01	0.01	0.05
10	N.S.	N.S.	0.05
11	0.01	0.05	0.01
15	N.S.	0.05	N.S.

N.S. — No significance
0.05 — F value exceeds 5% level, but is below 1% level
0.01 — F value exceeds 1% level.

10, and 15. The cortex is relatively heavier in the males when all 158 birds are considered, but lines 10 and 11 deviate from this pattern with a slightly heavier cortex in the female. The sex difference in the medulla is due to a heavier relative weight for the males in lines 6, 10, and 15, with equal values for the sexes in line 11. Line differences were highly significant for all organ weights after correction for total body size; families within lines differed only in total adrenal, adrenal cortex, and adrenal medulla weight. Within line analyses were made to determine the origin of the family differences observed in Table 5. The results of this analysis are given in Table 6, and indicate family differences in total adrenal in lines 6 and 11, in cortex in lines 6, 11, and 15, and in adrenal medulla in lines 6, 10, and 11.

The results of the analysis in Table 5 cannot be compared directly with the organ:body weight ratios given in Table 4. In computing organ:body weight ratios, heavier individuals are at a disadvantage, because of the ubiquitous inverse relationship of this ratio with body weight. Accordingly, covariance is a better procedure in that the

organs are compared with the mean body weight of the respective groups. In spite of this discrepance between the two methods, the ratios given are of some use in estimating where the significant differences occur, especially when consideration is given to the fact that ratios tend to underestimate the differences.

It is obvious that each of the lines has a distinctive growth pattern in respect to the organs studied. This difference extends to sexes within lines for the adrenal and pituitary, and to families within lines for the adrenal. The practice of inbreeding within these lines, together with the reasonably constant management procedures which have been followed indicate that the differences observed in the response of lines and families within lines are genetic in origin.

Organ-body weight and inter-organ correlations.—Different growth patterns for the organs within the respective lines necessitate computation of all correlations on a within line basis. Correlations for all lines combined also are given, although they are of value only when the individual line correlations are in agreement; r values for the individual lines are given in Table 7, and are based on relationships within individual birds; males and females have been combined. Liver, spleen, and adrenal cortex show positive significant correlations with body weight for all lines combined, and while the values of the individual lines differ, they all are positive. In other comparisons, as with total adrenal, adrenal medulla, pituitary, thymus, and pancreas, correlations with body weight may vary from highly significant and positive to negative. None of the negative correlations were significant, but it is obvious that the pooled correlations for these particular comparisons cannot at present be evaluated.

Inter-organ correlations with the effects of body weight removed are given in Table 8. A comparison of these values with those for the same organs given in Table 7 indicates the extent to which these relationships are determined by general growth factors.

Correlations of the liver with other organs have been reduced by correction for body weight to a non-significant value in comparison with total adrenal, adrenal cortex in line 11, and pancreas in line 11. The correlation of liver with adrenal cortex in line 6, though reduced in size, still is significant. However, some of the negative correlations have become significant, as the liver:adrenal cortex comparison for line 15, and the liver:thymus relationship for line 6. The liver:pituitary value was changed from a negative correlation of —0.388 with significance at the 5 percent level, to —0.719, which is significant at the 1 percent level.

TABLE 7

BODY:ORGAN WEIGHT AND INTRA-ORGAN CORRELATIONS WITHIN FAMILY AND SEX FOR 4 INBRED LINES OF WHITE LEGHORN CHICKENS

Organ	Line	d. f.	Total adrenal	Adrenal cortex	Adrenal medulla	Pituitary	Thymus	Spleen	Pancreas	Liver
Body	6	31	0.509**	0.529**	0.313	0.188	0.470**	0.447**	0.298	0.393*
	10	31	0.159	0.054	0.175	0.116	—0.145	0.114	—0.072	0.266
	11	29	0.536**	0.330	0.399*	—0.040	0.430*	0.452**	0.529**	0.637**
	15	29	—0.126	0.155	—0.037	—0.258	—0.015	0.586**	0.218	0.616**
All lines		123	0.319**	0.245**	0.236**	—0.119	0.156	0.375**	0.288**	0.460**
Liver	6	31	0.393*	0.569**	0.099	—0.103	—0.114	0.160	—0.057	
	10	31	—0.001	0.054	—0.084	—0.072	—0.143	0.204	0.175	
	11	29	0.430*	0.362*	0.234	0.030	0.199	0.194	0.429*	
	15	29	—0.263	—0.201	—0.184	—0.388*	—0.118	0.291	—0.051	
All lines		123	0.179*	0.243**	0.039	—0.134	—0.056	0.203*	0.144	
Pancreas	6	31	0.282	0.144	0.319	—0.362*	0.354*	0.372*		
	10	31	0.024	0.098	—0.029	—0.181	0.156	0.384*		
	11	29	0.447*	0.311	0.341	—0.006	0.093	0.405*		
	15	29	0.242	0.076	0.315	0.066	0.345	0.500**		
All lines		123	0.271**	0.169	0.273**	—0.106	0.222*	0.397**		
Spleen	6	31	0.158	0.142	0.125	—0.171	0.535**			
	10	31	—0.024	—0.048	—0.107	—0.194	0.304			
	11	29	0.366*	0.440*	0.129	—0.054	0.488**			
	15	29	—0.142	—0.183	—0.033	—0.282	0.359**			
All lines		123	0.106	0.106	0.041	—0.166	0.387**			
Thymus	6	31	0.023	—0.053	0.061	—0.244				
	10	31	0.251	0.085	0.286	0.009				
	11	29	0.229	0.142	0.234	—0.088				
	15	29	—0.266	—0.261	—0.182	0.207				
All lines		123	0.047	0.024	0.072	0.018				
Pituitary	6	31	—0.142	0.008	—0.259					
	10	31	0.114	0.161	—0.031					
	11	29	0.092	0.127	0.004					
	15	29	—0.223	—0.213	—0.164					
All lines		123	—0.067	0.002	—0.127					

* — Significant at 5% level
** — " " 1% "

TABLE 8

PARTIAL CORRELATIONS: INTRA-ORGAN COMPARISONS WITHIN FAMILY AND SEX OF TABLE 7 CORRECTED FOR BODY WEIGHT

Organ	Line	d. f.	Total adrenal	Adrenal cortex	Adrenal medulla	Pituitary	Thymus	Spleen	Pancreas
Liver	6	29	0.244	0.463**	—0.027	—0.032	—0.368*	—0.019	—0.198
	10	29	—0.045	0.041	—0.138	—0.107	—0.109	0.181	0.202
	11	27	0.136	0.209	—0.029	0.072	—0.108	0.137	0.141
	15	27	—0.237	—0.381*	—0.205	—0.719**	—0.138	0.110	—0.241
All lines		122	0.038	0.151	—0.081	—0.090	—0.146	0.037	0.014
Pancreas	6	29	0.159	—0.017	0.249	—0.326	0.254	0.280	
	10	29	0.036	0.102	—0.017	—0.174	0.147	0.396*	
	11	27	0.228	0.170	0.167	0.032	—0.176	0.219	
	15	27	0.222	0.044	0.331	0.130	0.357	0.471**	
All lines		122	0.197*	0.106	0.220*	—0.075	0.187*	0.326**	
Spleen	6	29	—0.090	—0.124	—0.018	—0.099	0.411*		
	10	29	—0.043	—0.055	—0.130	—0.210	0.326		
	11	27	0.164	0.345	—0.063	—0.040	0.365		
	15	27	—0.085	—0.342	—0.014	—0.167	0.454*		
All lines		122	—0.016	0.016	—0.053	—0.132	0.359**		
Thymus	6	29	—0.285	—0.403*	—0.103	—0.180			
	10	29	0.281	0.094	0.320	0.026			
	11	27	—0.002	0.000	0.075	0.078			
	15	27	—0.266	—0.262	—0.182	—0.218			
All lines		122	—0.003	—0.015	0.037	—0.037			
Pituitary	6	29	—0.055	0.129	—0.215				
	10	29	0.097	0.156	—0.052				
	11	27	0.134	0.149	0.022				
	15	27	—0.267	—0.181	—0.160				
All lines		122	—0.031	—0.028	—0.161				

Other inter-organ correlations dependent upon general body size are: pancreas and total adrenal in line 11, pancreas and thymus in line 6, pancreas and spleen in lines 6 and 11, possibly pancreas and pituitary in line 6, spleen and adrenal in line 6, spleen and adrenal cortex in line 11, and spleen and thymus in line 11. The following positive relationships have been increased: pancreas and spleen in line 6 and spleen and thymus in line 15. A significant negative correlation between adrenal cortex and thymus was obtained in line 6.

Correlations of the spleen with pancreas and thymus, and of pancreas with thymus, were not greatly affected by the covariance correction, and indicate independence of these organs from general growth patterns. However, partial regressions between spleen, pancreas, and thymus, when single organs are held constant, indicate that there are common factors of low value for all three organs. These factors explain the pancreas:thymus correlation. The relationships of the pancreas with the spleen and of the spleen with the thymus appear to have no common basis.

The pancreas:adrenal relationship is positive in all lines, and even though almost zero in line 10, the pooled value is significant and indicates common growth determinative factors for all lines.

A low degree of association between weights of most of the organs is indicated in Table 8. Where similar r values of like sign are observed in all lines, and the pooled correlation is significant, general biological relationships can be established. However, it is equally important that correlations between some organs are greatly influenced by genetic constitution, for r values as divergent as the 0.463 and —0.381 for liver:adrenal cortex comparisons in lines 6 and 15 may be obtained. Also, correction for body weight changes correlations in certain lines more than in others.

Discussion

The intermingling of cortical and medullary tissues of the chicken adrenal makes measurement by the customary weighing and planimeter procedures exceedingly laborious. The method described by Chalkley (1943), in which pointers at the focal plane of the ocular are used, gives a rapid accurate, estimation of relative volumes of tissues with this type of distribution. Increased accuracy over that obtained with a microprojector results primarily from increased resolving power and ability to eliminate connective tissues, blood vessels, and nerves.

Masses of cortical cells near the periphery of the adrenal appear

to be more regular in arrangement than those in the center of the gland. This situation also has been discussed by Müller (1929), and Sauer and Latimer (1931). Müller (1929) stated that the cortical masses also were larger near the periphery. However, the present data are in agreement with those of Sauer and Latimer in that cortex and medulla show the same percentage in all regions of the adrenal. For the population as a whole, the right and left adrenals show equal percentage composition, but this relationship does not necessarily hold for individual birds. The conclusion of Sauer and Latimer (1931) that there is a higher percentage of cortex in females is in agreement with the data for lines 10 and 11, but no sex differences were observed in lines 6 and 15. A higher percentage of medullary tissue was indicated for males of lines 10 and 15, with no difference in lines 6 and 11. It is obvious that sex differences in relative proportions of cortex and medulla of chickens are influenced by genetic factors. Weight of cortical and medullary tissue in relation to total body weight also is under genetic control as indicated by the significant line differences given in Table 5. Increase in medullary tissue was observed in the frizzled condition by Landauer and Aberle (1935). While the increase in medullary tissue is an indirect one, it, nevertheless, depends on genetic constitution. The data obtained in the present study give no clue as to the developmental bases for the genetic changes in quantitative histology of the adrenal.

Conflicting results have been reported on sex differences in pituitary and adrenal weights of some species. In chickens, Landauer and Aberle (1935) reported heavier pituitaries in male fowl; equal pituitary weights were reported for males and females by Juhn and Mitchell (1929). The data reported here are in agreement with the results of Landauer and Aberle (1935). Kibler et al. (1942) observed heavier adrenals in female rabbits, while Allanson (1932) failed to detect a sex difference. Rogers and Richter (1948) give data indicating that the consistently heavier adrenals in female Norway rats do not exist in Alexandrine rats. The current data indicate that differences in relative adrenal weight are markedly influenced by genetic mechanisms, and suggest that many of the discrepancies reported in the literature are the result of different genetic constitutions of stocks of experimental animals.

Correlations in Table 7 agree with those given by Oakberg and Lucas (1949) for body:pancreas weight and body:liver weight but are much lower for the liver:pancreas comparison. Also, a negative (but

not significant) body:spleen relationship was observed in the 600-day females (Oakberg and Lucas, 1949), while in Table 7 this correlation is positive and highly significant. The pancreas:spleen correlation is positive and highly significant for both groups of birds. As emphasized earlier in this paper, results on the different groups can be adequately evaluated only when they are in agreement. In comparing the data of Oakberg and Lucas (1949) with present results, the following differences in procedure cannot be evaluated: the present data are based on 120-day birds of both sexes, the earlier report was based on 600-day females; only 4 of the 10 lines used in the previous report appear in this analysis, and the pooled correlations of the lines represented may differ greatly; use of organ:body weight ratios (Oakberg and Lucas, 1949) discriminates against heavier individuals, and may lead to results somewhat different from those obtained by covariance; finally, samples from the same inbred lines may not be constant from year to year. Positive body:liver weight, body:pancreas weight, and pancreas:spleen weight correlations may be established as common relationships in the Laboratory flock.

The negative relationship which has been observed between adrenal cortex weight and weight of lymphoid organs under condition of stress (Selye, 1936, 1937, 1946; Dougherty and White, 1943, 1944, 1945, 1946, 1947) might be expected to exist in normal animals. Line 6 gave the only significant negative correlation between adrenal cortex and thymus, and in line 15 the correlation was negative but not significant. In lines 10 and 11 no relationship was observed. At present it appears as if the question must be left undecided. This is particularly true in view of the fact that amount of glandular tissue is not an accurate measure of gland activity. Large size may indicate either hyper- or hypofunction. Also, as emphasized by Gardner (1948) rate of hormone production, rate of hormone inactivation, response of target tissues, and quality of hormone produced must be considered. The pancreas-spleen correlation may be the result of hormone control similar to the alarm reaction, as Selye (1946) lists atrophy of the pancreas as a corollary of lymphoid tissue involution. This relationship, however, is questionable in view of the absence of a pancreas: thymus relationship. The factors common to pancreas, thymus. and spleen are in best agreement with pituitary adrenal cortex control, but these factors are of minor importance in the present material.

Most of the inter-organ correlations are small, and while additional data or use of different age groups might establish significant relation-

ships in more comparisons, the value of the correlations probably would remain low. The most important fact appears to be the effect of lines on these relationships. Asmundson and Lerner (1933) also observed differences in growth between different strains of Leghorns, and in a later paper (Asmundson and Lerner, 1934) they demonstrated that growth differences may exist only for a portion of the growing period. The influence of this condition on the present data cannot be determined.

Inbred lines provide excellent tools for the study of genetic control over growth relationships. However, extrapolation of the results to a condition which may be considered normal is decidedly limited, and relationships can be established only when all lines behave alike.

Summary

Quantitative histology of the adrenals and body and organ weights were studied for 158, 120 day, male and female White Leghorn chickens from 4 inbred lines.

Significant differences in percentages of adrenal cortex and medulla were observed between birds of the same family and sex in all lines. Sex differences were demonstrated for percentage of cortex and medulla in one line, in percentage of medulla for a second line, and in percentage of cortex for a third line.

In relation to total body weight, heavier adrenals were observed in the male for lines 6, 10, and 15, with the female adrenal relatively heavier in line 11. Family differences in relative weight of the total adrenal, adrenal cortex, and adrenal medulla were observed in some lines but not in others.

Inter-organ correlations usually were reduced when corrected for body weight, but specific relationships independent of general growth also were indicated. Positive correlations between pancreas and spleen and between spleen and thymus were obtained. The two correlations, however, do not arise from common factors.

The data are discussed in relation to the growth patterns of the respective lines, and from the variation in correlations it is concluded that genetic constitution is an important variable in all the comparisons. Implications of line differences are emphasized in establishment of physiological relationships in growth of organs and tissues of the domestic fowl.

Acknowledgments

The cooperation of the staff of the U. S. Regional Poultry Research Laboratory throughout this study has been greatly appreciated, and especially the help and criticism given by Dr. Alfred M. Lucas. Dr. Leo Katz of the Mathematics Department of Michigan State College contributed many suggestions relative to statistical procedures.

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Growth, 1951, **15**, 79-100.

INFLUENCE OF GENETIC CONSTITUTION ON GROWTH OF LYMPHOID TISSUE IN LIVER AND PANCREAS OF WHITE LEGHORN CHICKENS, AND CORRELATION OF LYMPHOID TISSUES WITH WEIGHTS OF SOME VISCERAL AND ENDOCRINE ORGANS[1]

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Considerable interest has centered recently on the function of the pituitary and adrenal cortex in growth and maintenance of lymphoid tissues. Reviews of the literature and development of this concept have been given by Dougherty and White (1944, 1945, 1946, 1947), Jones (1949), Li and Evans (1947), Selye (1936, 1937, 1946), Skelton (1950), and Zeckwer (1948). The general physiological relationship of pituitary, adrenal cortex, and lymphoid tissues appears to be well established. The inhibition of tissue growth by adrenal cortical hormones has been extended to include cartilage, bone, and bone marrow (Baker and Ingle, 1948), while presence of the adrenal has been shown to be necessary for normal maintenance of the reticulo-endothelial system (Gordon and Katsh, 1949). Inhibition of certain tumors by hormones of the adrenal cortex has been reported for leukemia in mice (Law, 1947; Law and Speirs, 1947), leukemia in rats (Murphy and Sturm, 1943, 1944, 1950; Sturm and Murphy, 1944), and sarcomas in mice (Diller, et al., 1948). That the adrenal reacts to certain tumors as in general stress is indicated by adrenal hypertrophy in tumor-bearing animals (Murphy and Sturm, 1948; Savard, 1948).

[1]This work was made possible by a grant from Swift and Company, Chicago, Illinois, to the Agricultural Experiment Station of Michigan State College. Laboratory facilities, equipment, and materials were furnished by the U. S. Regional Poultry Research Laboratory.

Journal Article No. 1259 of the Michigan Agricultural Experiment Station, East Lansing, Michigan.

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Differences in development of lymphoid tissues that suggest a relationship with the adrenal cortex have been observed in wild and domestic rats, with the wild animals having heavier adrenals and fewer Peyer's patches (Richter and Hall, 1947). Likewise, Stein and Cheng (1948) observed a negative correlation between adrenal size and total leucocyte counts in inbred strains of mice. Casey and Pearce (1931) obtained a negative relationship between leucocyte counts in rabbits and resistance to an inoculated tumor, and Kelsall (1946) observed a positive correlation between number of Peyer's patches and incidence of spontaneous mammary carcinoma in mice. The results of Casey and Pearce (1931) and Kelsall (1946) may involve differences in the adrenal cortex or its secretions.

Establishment of characteristic levels of physiological activity of a gland is at present a difficult undertaking. As emphasized by Gardner (1948), amount of hormone produced, capacity of the tissues to respond, rate of hormone destruction, and quality of hormone produced must be considered. Various combinations of these properties could account for the genetic characteristics of an individual. The best established differences among strains of animals concern their response to endocrine secretions (Bates, et al., 1941; Munro, et al., 1943; and Gardner, et al., 1944).

Quantitative studies of lymphoid tissues in the pancreas of chickens in relation to genetic constitution and incidence of lymphomatosis (Lucas, 1949a; Lucas and Oakberg, 1950), and in relation to age and sex (Oakberg and Lucas, 1949), have been made. Growth changes in lymphoid tissues in some of the splanchnic nerves also have been determined for the 1-300-day period after hatching (Oakberg, 1950). In both pancreas and splanchnic nerves, a peak in percentage of lymphoid tissue was observed for the 40- and 100-day groups, but the significance of higher values at these ages cannot be considered as established due to the small number of birds involved. Nevertheless, these results correspond with the higher relative development of germinal centers in children and their regression in adults (Hwang, et al., 1938; Denz, 1947), growth and atrophy of the thymus (Hammar, 1921a, b; Boyd, 1932), growth of lymphoid tissues of the rabbit (Hellman, 1914), regression of Malpighian follicles in senile rats (Andrew, 1946), and age changes in deep cervical lymph nodes of rats (Andrew and Andrew, 1948).

In view of the large differences in quantitative composition of the

adrenal and in relative weight of the adrenal cortex, together with the importance of adrenal cortex in regulation of lymphoid tissues, it would be of interest to determine what relationships exist between weight of cortical tissue of the adrenal and amount of ectopic lymphoid tissue in the liver and pancreas of chickens. Gross weight may not be an adequate measure of gland function because the total interaction of various factors undoubtedly is involved, nevertheless, utilization of inbred lines maintained under reasonably constant environmental conditions should indicate the effect of genetic constitution on total growth patterns of the organs and tissues studied.

Materials and Methods

Samples of liver and pancreas for this study were obtained from the same 158 birds used in organ:body-weight and inter-organ comparisons reported by Oakberg (in press). Of the four inbred lines, 6 and 10 have been selected for resistance, and 11 and 15 have been selected for susceptibility to lymphomatosis (Waters, 1945).

Three slices of tissue were taken from the right lobe of each liver: one near the tip, one at the level of the gall bladder, and one above the gall bladder. Blocks of pancreas were taken at about ¼, ½, and ¾ the length of the gland. All tissues were fixed in Zenker-formol, embedded in paraffin, sectioned at 5μ, and stained in hematoxylin azure II—eosin. One section from each block was studied.

Large section size and numerous lymphoid areas made utilization of entire liver sections impractical. Accordingly, a small rubber stamp which outlined an area of about 9.8 sq. mm. was used for sampling liver tissue. The tips of each section were superimposed upon the outer lines of a drawing of a wedge which was divided into 10 equal sectors. Three of these sectors were selected by a table of random numbers, and areas were stamped in the following sequence as the sectors were chosen: left margin, center, and right margin of the section. Data were pooled to form a single estimate for the section. As a check of randomly chosen areas indicated a standard deviation of only ± 0.37 sq. mm., the area sampled was considered uniform for each bird. Larger blood vessels were avoided when the areas were stamped. Projected images of all lymphoid and eosinophil areas were traced and measured with a planimeter. All readings were converted to square millimeters.

Foci of eosinophils, lymphoid cells, and a mixture of eosinophilic

and lymphoid cells occur in chicken livers. Most areas were either primarily eosinophilic (probably myelocytic) or lymphoid in nature. However, in some cases, separation was somewhat arbitrary. These mixed areas were classified on the basis of primary cell type; when sufficiently discrete, islands of specific cells were removed from accumulations of the opposite type and treated as separate areas.

All lymphoid areas in pancreas sections were traced and measured. As the sample size was not constant, total section area also was determined.

Data for number of foci, size of individual areas, and total tissue were highly skewed both for lymphoid and eosinophilic tissues. Use of the log of $1 + X$ (Bartlett, 1947) gave a close approximation to a straight line when plotted against probits of cumulative class frequencies for all variables. Accordingly, this transformation has been used throughout in analysis of the data. Covariance was used to remove family and sex variation from all correlations.

Results

Mean number, size of lymphoid foci in $mm.^2$, and percentage of lymphoid tissue are given by family and sex in Table 1. The variation in all measurements is marked, and differences between liver and pancreas are particularly conspicuous, with a mean percentage of 0.407 for the liver, and 0.244 for the pancreas. This difference results from the number of areas, for the mean size of 0.0139 $mm.^2$ for lymphoid foci in the pancreas is three times the value of 0.0046 $mm.^2$ observed for the liver.

Liver

Lymphoid tissues.—Line differences were highly significant for all measurements of lymphoid foci in the liver (Table 2). Sex differences in size of lymphoid areas were observed in lines 6 and 11, with significance at the 5 and 1 per cent levels, respectively. Females had the larger means in both cases. Also, females had a higher percentage in line 15, with the difference significant at the 5 per cent level. Families within lines differ in size and mean area of lymphoid foci and in percentage of lymphoid tissue in lines 6 and 11, with no family differences apparent in lines 10 and 15. Birds within family and sex showed great variation, and these differences were substantiated by the significant differences obtained for all comparisons except size

TABLE 1

SEX BY FAMILY MEANS FOR NUMBER AND SIZE OF INDIVIDUAL LYMPHOID AREAS AND TOTAL LYMPHOID TISSUE IN THE LIVER AND PANCREAS OF 4 INBRED LINES OF WHITE LEGHORN CHICKENS

		Liver						Pancreas					
		Number of areas		Mean size of areas in mm²		Percentage of lymphoid tissue		Number of areas		Mean size of areas in mm²		Percentage of lymphoid tissue	
Line	Family	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
6	257	32.2	38.2	.0039	.0053	.213	.346	11.0	9.8	.0144	.0167	.381	.468
	262	67.2	54.4	.0041	.0043	.466	.395	4.2	5.0	.0111	.0197	.110	.241
	269	53.8	48.8	.0050	.0061	.452	.507	8.8	6.6	.0372	.0176	.782	.291
	285	43.2	59.0	.0041	.0052	.299	.525	4.2	5.4	.0072	.0164	.121	.234
Line x sex means		49.1	50.1	.0043	.0052	.358	.443	7.1	6.7	.0175	.0176	.349	.309
Line means			49.6		.0048		.401		6.9		.0176		.329
10	461	32.6	42.0	.0037	.0035	.205	.251	7.8	7.8	.0124	.0185	.167	.292
	462	24.4	39.8	.0040	.0034	.155	.230	7.4	6.4	.0105	.0104	.173	.138
	470	29.6	41.8	.0031	.0035	.156	.249	7.8	6.4	.0108*	.0107*	.154	.142
	491	24.4	34.6	.0035	.0043	.146	.254	5.0	8.0	.0091	.0115	.094	.179
Line x sex means		27.8	39.6	.0036	.0037	.166	.246	7.0	7.2	.0107	.0128	.147	.188
Line means			33.7		.0037		.206		7.1		.0118		.168
11	505	55.0	51.4	.0045	.0049	.423	.427	8.4	5.4	.0139	.0156	.186	.164
	519	59.0	78.8	.0088	.0069	.883	.922	16.0	15.0	.0185	.0173	.522	.436
	520	68.0	86.4	.0029	.0068	.443	.990	4.2	7.4	.0159	.0121	.166	.162
	528	43.5	67.3*	.0032*	.0037*	.239*	.428*	10.5*	5.7*	.0113**	.0105**	.214*	.117*
Line x sex means		56.4	71.0	.0049	.0056	.497	.692	9.8	8.4	.0149	.0139	.272	.220
Line means			63.7		.0053		.595		9.1		.0144		.246
15	707	44.6	45.4	.0041	.0050	.313	.383	6.2	10.8	.0114	.0130	.150	.307
	709	56.8	58.6	.0043	.0044	.413	.439	8.6	9.0	.0126	.0083	.242	.164
	715	51.4	58.0	.0055	.0049	.476	.485	10.2	14.2	.0134	.0160	.260	.446
	725	36.3	74.0*	.0046*	.0047*	.281*	.596*	7.2*	5.0*	.0115*	.0092*	.186*	.101*
Line x sex means		47.3	59.0	.0046	.0048	.371	.476	8.1	9.8	.0122	.0116	.210	.255
Line means			53.2		.0047		.424		9.0		.0119		.233
General means			50.1		.0046		.407		8.0		.0139		.244

All means given are on a bird basis, on a total of 3 sections.

* Based on four birds; ** based on 3 birds; all other means based on 5 individuals.

TABLLE 2

SUMMARY OF RESULTS OF VARIANCE ANALYSIS OF DATA IN TABLES 1 AND 3. BASED ON LOG 1 + x

	Lymphoid tissues						Eosinophil tissues		
	Liver			Pancreas					
	Number areas	Size of areas in mm.^{2}	Total lymphoid tissues	Number areas	Size of areas in mm.^{2}	Per cent lymphoid tissue	Number areas	Size of areas in mm.^{2}	Total eosinophil areas
Line 6									
Sex	N.S.	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.05	N.S
Families	0.01	0.01	0.05	0.01	0.05	N.S.	0.01	N.S.	0.01
Birds within family and sex	0.01	0.01	0.01	0.01	N.S.	N.S.	0.01	N.S.	0.01
Sections within birds		0.01			N.S.			0.01	
Line 10									
Sex	0.05	N.S.	0.05	N.S.	N.S.	N.S.			
Families	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.			
Birds within family and sex	0.01	N.S.	0.05	0.01	0.01	0.01			
Sections within birds		N.S.			N.S.				
Line 11									
Sex	N.S.	0.01	0.05	N.S.	N.S.	N.S.			
Families	0.05	0.01	0.01	0.01	N.S.	0.01			
Birds within family and sex	0.01	0.01	0.01	0.01	N.S.	0.05			
Sections within birds		0.01			N.S.				
Line 15									
Sex	N.S.	N.S.	0.05	N.S.	N.S.	N.S.			
Families	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.			
Birds within family and sex	0.01	0.01	0.01	0.01	N.S.	N.S.			
Sections within birds		N.S.			N.S.				
Line comparisons	0.01	0.01	0.01	N.S.	0.05	N.S.			

N.S.—No significance.
0.05—F value significant at 5% level.
0.01—F value significant at 1% level.

of lymphoid areas in line 10. The significant differences observed for size of areas between sections within birds (lines 6 and 11) are difficult to explain. Either sampling errors or unequal distribution of lymphoid tissues in individual birds would give these results. There is some subjective evidence for unequal distribution of lymphoid foci, but it is a property of individual birds and not characteristic of the portion of the liver from which the sections were taken, for no significant differences were obtained between regions when all birds within a line were combined.

Eosinophilic tissues.—One of the most striking line differences in this material was the presence of foci of eosinophilic cells in the liver of line 6. The cells comprising these areas were not subjected to extensive study, but most of them had round granules, usually an oval nucleus, and appeared to be similar to the immature granulocytes described by Jordan (1936). Similar foci were present in lines 10, 11, and 15, but their occurrence was very irregular and of insufficient extent for quantitative evaluation. Accordingly, data for line 6 only are given in Table 3, with results of variance analyses in Table 2.

TABLE 3

SEX BY FAMILY MEANS FOR NUMBER, SIZE OF INDIVIDUAL AREAS, AND PERCENTAGE OF EOSINOPHIL TISSUE IN THE LIVER OF LINE 6

Family	Number of areas		Mean size of areas in mm.^{2}		Per cent eosinophil tissue	
	Males	Females	Males	Females	Males	Females
257	30.8	17.2	.0028	.0021	.164	.063
262	14.0	18.0	.0021	.0028	.050	.086
269	11.0	13.8	.0027	.0021	.050	.049
285	8.4	8.6	.0019	.0021	.027	.031
Line x sex means	16.1	14.4	.0024	.0023	.073	.057
Line means		15.3		.0024		.065

Mean size of areas was slightly larger in males, with the difference significant at the 5 per cent level. Families and birds within family and sex differed greatly, with significance at the 1 per cent level for both number of areas and total area of eosinophilic tissue. Mean size of areas differed between sections within birds. The type of distribution curves and results of statistical analyses show the same type of variations that have been observed for lymphoid tissues. However, the two types of tissues appear to have no common relationships which could be detected with these data, for no correlations

of eosinophil areas with lymphoid tissues of either liver or pancreas were obtained (Table 4). However, common factors for formation of new areas and their subsequent growth were indicated with an r of 0.620 between number and mean area of eosinophil foci.

Pancreas

Lymphoid tissue.—Fewer significant differences in lymphoid tissues were observed in the pancreas than in the liver (Table 2). Lines differed only in mean size of areas. No sex effects were observed. Family differences were obtained for number of areas in lines 6 and 11, size of areas in line 6, and percentage of lymphoid tissue in line 11. Birds within family and sex differed in number of areas in all lines, and in percentage of lymphoid tissue in lines 10 and 11. Failure to detect as many differences in the pancreas as in the liver probably resulted from larger number of areas sampled in the liver, thereby giving more accurate estimates, and the correspondingly high error variances obtained for data on the pancreas. Effect of section size on amount of lymphoid tissue was estimated by correlation of total lymphoid tissue area with section area. No correlation was observed in lines 6 and 10, and positive r values significant at the 5 per cent level were obtained in lines 11 and 15. Correlations between total lymphoid tissue and percentage of lymphoid tissue (Table 4) indicate that no serious error would result in lines 6, 10, and 11 if no correction were made for section size. In line 15 the amount of pancreatic tissue must be considered.

Correlation of Lymphoid Foci in Liver and Pancreas

If all lymphoid tissues of the body are under a common regulatory mechanism, particularly if this mechanism is humoral in nature, a correlation of lymphoid tissues in different regions of the body should result. Correlations between lymphoid areas and total lymphoid tissues of liver and pancreas are given in Table 4. Computations were based both on total lymphoid tissue and on percentage data. As expected, use of percentages results in almost no change in the values obtained for the liver comparisons, but does result in a slight change in correlations with the pancreas. For the pancreas the percentage basis probably is the better measurement.

A significant correlation between total lymphoid tissue of the liver and pancreas was observed only in line 10; this relationship appears

TABLE 4

WITHIN BIRD CORRELATIONS FOR LYMPHOID TISSUES OF LIVER AND PANCREAS AND EOSINOPHIL TISSUES OF LIVER

TABLE 4

WITHIN BIRD CORRELATIONS FOR LYMPHOID TISSUES OF LIVER AND PANCREAS AND EOSINOPHIL TISSUES OF LIVER

				Lymphoid tissue, pancreas				Lymphoid tissue, liver		Eosinophil tissue, liver		
		Line	d.f.	Per cent lymphoid tissue	Mean size of areas in mm.^{2}	Number of areas	Total lymphoid tissue	Mean size of areas in mm.^{2}	Number of areas	Mean size of areas in mm.^{2}	Number of areas	Total eosinophil tissue
Lymphoid tissue in liver	Total lymphoid tissue	6	31	0.011	—0.037	0.064	—0.026	0.643**	0.715**	0.163	—0.046	0.009
		10	31	0.313	0.071	0.344*	0.354**	0.714**	0.852**			
		11	29	0.160	0.316	0.087	0.056	0.769**	0.858**			
		15	29	0.039	0.064	—0.014	—0.018	0.707**	0.895**			
	Number areas	6	31	—0.038	—0.136	0.004	—0.085	0.096		0.216	0.140	0.172
		10	31	0.303	—0.079	0.361*	0.368*	0.297				
		11	29	0.076	0.312	0.030	—0.002	0.333				
		15	29	—0.019	0.116	—0.111	—0.075	0.318				
	Size of areas	6	31	0.212	0.002	0.356**	0.202			0.019	—0.216	—0.181
		10	31	0.203	0.121	0.168	0.202					
		11	29	0.193	0.158	0.115	0.098					
		15	29	0.127	—0.033	0.151	0.094					
	Per cent lymphoid tissue	6	31	0.097								
		10	31	0.314								
		11	29	0.161								
		15	29	0.034								
Lymphoid tissue in pancreas	Total lyhphoid tissue	6	31	0.902**	0.660**	0.817**				0.183	0.061	0.110
		10	31	0.977**	0.808**	0.841**						
		11	29	0.933**	0.695**	0.843**						
		15	29	0.600**	0.409*	0.510**						
	Number areas	6	31	0.829**	0.297					0.168	—0.001	0.040
		10	31	0.787**	0.368*							
		11	29	0.784**	0.201							
		15	29	0.776**	0.047							
	Size of areas	6	31	0.753**						0.224	—0.025	0.081
		10	31	0.828**								
		11	29	0.652**								
		15	29	0.634**								
Eosinophil tissue in liver	Total eosinophil tissue	6	31							0.814**	0.952**	
	Number areas	6	31							0.620**		

*Significant at 5% level.
**Significant at 1% level.
Underlined values have 1 less degree of freedom.

to depend upon number of areas. In line 6 a positive correlation between size of areas in the liver and number of areas in the pancreas was obtained. Some of the correlations, while small, nevertheless appear to be samples from a homogeneous population. Pooling of all lines to obtain a total of 125 degrees of freedom for these comparisons results in an r of 0.187 (significant at the 5% level) for size of areas in the liver with percentage of lymphoid tissue in the pancreas, an r of 0.207 (significant at the 5% level) for size of areas in the liver with number of areas in the pancreas, and a nonsignificant value of 0.161 for percentage of lymphoid tissue in the liver with percentage of lymphoid tissue in the pancreas. Thus, there appears to be some relationship between size of lymphoid foci in the liver and percentage of lymphoid tissue in the pancreas; specifically, this correlation depends upon the association between size of areas in the liver with number of areas in the pancreas. Relationship between the two organs is, however, quite low in these data.

Within organs, a positive relationship between number and size of lymphoid areas appears to exist. While the correlations are low and much variability remains unexplained, pooled estimates with 125 degrees of freedom gave an r of 0.241 (significant at the 1 per cent level) for number and size of lymphoid areas in the liver, and 0.191 (significant at the 5 per cent level) for the pancreas. Even though the correlations are low, some similar factors must be involved both in the origin of new lymphoid areas and their subsequent growth within an individual organ.

Correlations of Lymphoid and Eosinophil Tissues with Organ Weights

Correlations were computed for percentage of lymphoid and eosinophil tissues, number of individual areas, and mean size of areas with organ weights (Table 5). All correlations have been corrected for body weight. In general, the relationships are quite low with relatively few values reaching significant levels. Line differences are marked. For example, in lines 6 and 10 a positive relationship between percentage of lymphoid tissue in the liver and weight of pancreas and spleen is indicated, but no correlation exists in lines 11 and 15. In fact, the r with pancreas weight in line 11 is negative. A complete discussion of the results will not be given, as Table 5 is self-explanatory. However, those associations which appear to be common for all lines will be considered.

TABLE 5

WITHIN BIRD CORRELATIONS FOR LYMPHOID TISSUES OF LIVER AND PANCREAS AND EOSINOPHIL TISSUES OF LIVER WITH ORGAN WEIGHTS. EFFECT OF BODY WEIGHT ADJUSTED BY COVARIANCE

				Organ weights							
		Line	Degrees of freedom	Liver	Pancreas	Spleen	Thymus	Adrenal	Adrenal cortex	Adrenal medulla	Pituitary
Lymphoid tissue in liver	Number of areas	6	29	0.031	0.298	0.312	0.039	0.222	0.204	0.159	—0.178
		10	29	—0.028	0.539**	0.461**	0.226	0.326	0.452**	0.043	—0.131
		11	27	—0.085	—0.018	0.0002	0.376*	0.237	0.131	0.192	0.151
		15	27	—0.318	0.072	—0.131	0.239	—0.007	—0.138	—0.012	0.354
	Size of areas	6	29	0.001	0.320	0.347	0.110	—0.120	—0.017	—0.168	—0.119
		10	29	0.141	0.151	0.189	0.079	0.224	0.249	0.081	—0.041
		11	27	—0.338	—0.403*	0.138	0.138	—0.167	0.017	0.228	0.210
		15	27	—0.260	0.080	0.132	0.107	0.127	0.077	0.116	0.129
	Percentage lymphoid tissue	6	29	0.010	0.378*	0.394*	0.086	0.067	—0.121	—0.010	—0.179
		10	29	0.012	0.443*	0.443*	0.149	0.388*	0.464**	0.126	—0.106
		11	27	—0.246	—0.232	0.076	0.332	0.068	0.099	0.004	0.221
		15	27	—0.381*	0.125	0.007	0.292	0.022	—0.086	0.025	0.304
Lymphoid tissue in pancreas	Number of areas	6	29	0.117	0.224	0.218	—0.190	0.126	—0.033	0.211	—0.175
		10	28	—0.075	0.431*	0.328	—0.276	0.087	0.104	0.070	0.042
		11	27	0.048	0.206	0.210	0.234	—0.133	0.002	—0.140	0.371*
		15	27	—0.343	0.161	0.133	—0.117	0.249	0.048	0.327	0.190
	Size of areas	6	29	0.186	0.012	0.202	0.147	0.102	0.120	0.028	—0.085
		10	28	0.045	0.005	0.119	—0.004	0.120	0.063	0.134	0.030
		11	26	—0.165	0.006	—0.097	0.061	—0.179	—0.129	—0.117	—0.098
		15	27	0.154	—0.095	—0.065	—0.112	0.228	0.248	0.166	0.041
	Percentage lymphoid tissue	6	29	0.220	0.067	0.198	—0.093	0.164	0.065	0.176	—0.171
		10	29	0.006	0.163	0.364*	0.253	0.182	0.143	0.152	0.005
		11	27	—0.100	0.254	0.047	0.072	—0.249	—0.073	—0.188	0.234
		15	27	—0.115	0.005	—0.005	—0.154	0.296	0.077	0.337	0.126
Eosinophil tissue in liver	Number of areas	6	29	—0.016	0.362*	—0.135	—0.003	0.115	0.159	0.054	—0.132
	Size of areas	6	29	0.268	0.471**	0.149	0.042	0.320	0.241	0.272	—0.302
	Percentage eosinophil tissues	6	29	0.084	0.438*	—0.063	0.020	0.202	0.165	0.173	—0.179

*Significant at 5% level.
**Significnt at 1% level.

Results for all lines were pooled for those comparisons where similar relationships were indicated. On this basis, percentage of lymphoid tissue in the liver was correlated with spleen weight ($r = 0.259^{**}$, 122 d.f.) and thymus weight ($r = 0.235^{**}$, 122 d.f.). These correlations appear to depend upon number of areas for the thymus comparison ($r = 0.220^{*}$, 122 d.f.) and on size of areas for the spleen relationship ($r = 0.223^{*}$, 122 d.f.) (* = significant at 5% level, ** = significant at 1% level). Thus growth of lymphoid tissues in the liver shows a low but significant relation to growth of other lymphoid organs of the body.

Number of lymphoid areas of the pancreas were correlated with spleen ($r = 0.241^{**}$, d.f. 122) and pancreas ($r = 0.260^{**}$, d.f. 122) weights. Thus number of areas in the pancreas shows a relation with spleen in agreement with results of percentage of lymphoid tissue of the liver. However, relationship of the thymus to growth of lymphoid tissues in the pancreas differs from that shown by similar lymphoid structures in the liver. Also, it is apparent that most correlations with lymphoid tissue of the pancreas depend upon the basis of number, and that all r values are greatly reduced when computed on a percentage basis. Thus the relation of number of lymphoid areas to size of the pancreas becomes an important question. Whether both pancreas weight and number of lymphoid areas are responses to a common mechanism, or whether more lymphoid foci are a secondary effect of increased pancreas size cannot be determined.

Percentage of eosinophil tissue of the liver shows correlations different from those observed for lymphoid tissues in giving a rather high relationship with total body size. Correlations with pancreas weights are much higher. While it would be hazardous to reach a conclusion on the basis of one sample from a single line, it nevertheless appears as if eosinophil areas of the liver are more closely related to total growth patterns than are lymphoid foci.

Correlation of Percentage of Mortality with Lymphoid Tissues of Liver and Pancreas

A comparison of percentage of mortality from lymphomatosis with percentage of lymphoid tissue in the pancreas of females surviving to 600 days was made by Lucas and Oakberg (1950) and significant positive correlations between these variables were observed both on a family and on a line basis. This observation has been checked

TABLE 6

CORRELATION OF 600-DAY MORTALITY RECORDS WITH AMOUNT OF LYMPHOID TISSUE IN LIVER AND PANCREAS OF 120-DAY SIBS. BASED ON AVERAGES OF FAMILIES WITHIN LINES

	Correlations obtained with uncorrected mortality percentages			Correlations obtained with angles of the mortality percentages		
	Per cent lymphomatosis	Per cent "other mortality"	Per cent survivors	Per cent lymphomatosis	Per cent "other mortality"	Per cent survivors
Liver						
Number of lymphoid areas	—0.402	—0.295	0.693*	—0.400	—0.210	0.682*
Size of lymphoid areas	0.001	0.219	—0.193	0.002	0.172	—0.187
Per cent lymphoid tissue	—0.460	—0.125	0.610	—0.470	—0.131	0.595
Pancreas						
Number of lymphoid areas	0.752*	—0.137	—0.655	0.735*	—0.321	—0.665*
Size of lymphoid areas	0.265	—0.583	0.253	0.251	—0.578	0.242
Per cent lymphoid tissue	0.714*	—0.357	—0.416	0.700*	—0.506	—0.429

All correlations have 7 degrees of freedom.
*Significant at the 5 per cent level.

by the present data where percentage of lymphoid tissue of the pancreas of 120-day females was significantly correlated with incidence of lymphomatosis in sibs kept for 600 days ($r = 0.700$, Table 6). It is apparent that utilization of number alone gives almost the same correlation, with an r of 0.735. As data on lymphoid tissues given in Table 6 are based on 120-day birds, they should represent a more reliable sample of the various families than would be obtained with 600-day survivors. However, the present data include the undetermined variable of separate maintenance of the chickens used for histological study from those used for estimation of mortality rates. Unfortunately, line 11 was discontinued at this Laboratory during progress of the experiment, and family 470 of line 10 also was not kept for 600-day mortality records. This left only seven degrees of freedom for correlation coefficients based on families within lines. As only 3 lines were represented, no estimates were made on a line basis. Results based both on uncorrected percentage data and on angle transformations have been included in Table 6 in order that the close similarity of results from the two procedures may be demonstrated. Discussion of Table 6, however, has been limited to correlations obtained with angle transformations of mortality percentages.

Discrepancies between the results of Lucas and Oakberg (1950) and the present data in correlations between lymphoid tissues and "other mortality" may be due to sampling errors. The values reported previously were all positive and low, and while the current data indicate a negative relationship, the correlation coefficients are not large enough to be statistically significant.

Results obtained for comparison of lymphoid tissues of the liver with mortality percentages should be checked on a more extensive sample, for the results are the opposite of those observed for the pancreas. While not significant, the negative correlations of number of lymphoid areas and percentage of lymphoid tissues with percentage of lymphomatosis, —0.400 and —0.470, respectively, 7 degrees of freedom, are suggestive of a possible relationship which could be established with sufficient information. The combined negative values for number of areas with lymphomatosis and with "other mortality" give a significant, positive correlation of 0.682 with percentage of survivors. This is almost the same magnitude as the correlation of —0.665 observed between percentage of survivors and number of lymphoid foci in the pancreas. Similar contrasts are obtained

when size of lymphoid areas and percentage of lymphoid tissue are compared with survivors, but the correlations are not significant. Due to the small number of observations, only those correlations which agree with previous observations can be emphasized. However, the high values obtained for other comparisons, and especially those involving lymphoid tissues of the liver, indicate possible relationships which should be studied further.

Discussion

Growth of lymphoid tissues in the liver and pancreas of chickens definitely is influenced by genetic constitution. The different lines are uniform in showing a low but significant correlation between number and size of lymphoid areas within both liver and pancreas. Thus, formation of new areas and growth of these areas appear to depend upon some common mechanisms. On this basis, a higher correlation between lymphoid tissues of liver and pancreas might have been expected. Failure to observe a more definite relationship between the two organs may have resulted from large sampling variation in the pancreas, or all the areas measured in the liver may not have been comparable to those in the pancreas. Also, it has been observed in man (Young and Turnbull, 1931) that development of lymphoid tissue in different regions of the body is not necessarily correlated. Likewise, no relationships between lymphoid areas in the pancreas and in splanchnic nerves was observed in chickens (Oakberg, 1950). The importance of local factors exclusive of the general growth pattern is indicated by the low correlations observed in all between-organ comparisons of lymphoid tissues.

Correlation coefficients given in Table 6 suggest that not all lymphoid tissues sampled are the same. Ability to survive appears to be lowered by increasing amounts of lymphoid tissue in the pancreas, while for the liver the reverse situation obtains. In consideration of mortality, a positive correlation exists between percentage of birds dying from lymphoid tumors and number of lymphoid foci and with percentage of lymphoid tissue of the pancreas. Amounts of similar ectopic lymphoid areas in the liver tend to show negative correlations with incidence of lymphomatosis. This is especially puzzling in view of the fact that most cases of visceral lymphomatosis show liver involvement. The difference in results obtained on liver and pancreas could arise from several factors, but the most obvious are: (1)

lymphoid foci in the pancreas are abnormal and represent early stages in the development of lymphoid tumors, while those in the liver are normal hemopoietic tissues; (2) lymphoid foci are of the same type both in liver and pancreas, the difference observed is an organ effect; and (3) only one type of lymphoid area, the early stages in tumor formation, occurs in the pancreas while two or more types occur in the liver, some of which are early stages in tumorigenesis and others which are normal structures. In the latter hypothesis, it may be expected that in apparently normal birds the normal foci would predominate, and thereby may very well mask the frequency and total mass of abnormal lymphoid foci. Any of the three postulates given leads to many implications; the more obvious concern the evaluation of the physiological function of extra-vascular lymphoid foci.

The significance of lymphoid areas in the pancreas of ducks, turkeys, doves, and chickens recently has been discussed by Lucas (1949a, 1949b, 1950, 1951), Lucas and Oakberg (1950), Oakberg (1950), and Oakberg and Lucas (1949). In general, the normality of these areas has been questioned, particularly on the basis of destruction of adjacent tissues. This would appear to be especially true for splanchnic nerves of chickens (Oakberg, 1950). However, the present data indicate that extrapolation of these results to other portions of the body may not be justified, or at least not until better methods of separation of apparently identical lymphoid foci are available. Presence of numerous lymphoid areas in the liver appears to enhance probability of survival—an observation which should be explored further. Also, age changes in lymphoid tissues similar to those reported by Oakberg and Lucas (1949), and Oakberg (1950), should be continued, for while the peak in lymphoid tissue observed at 40 and 100 days in the pancreas and visceral nerves of chickens was thought to result from sampling errors, these data agree reasonably well with observations of Boyd (1932), Denz (1947), and Hwang et al. (1938) on man; Andrew (1946), Andrew and Andrew (1948) on rats, and Hellman (1914) on rabbits. Likewise, Jordan (1937) reported the regression of lymphoid nodules in the bone marrow of pigeons, turkeys, and chickens with increasing age.

Are myelocytic and lymphoid foci in the livers of chickens a reaction to an abnormal condition, possibly even to the presence of tumors elsewhere in the body as has been observed by Kelsall and Crabb (1947) in hamsters, or do they represent an intermediary stage in

the evolution of the lymph node? This complex problem still remains unsolved, but the phylogenetic approach of Jordan (1935, 1936) appears to have considerable merit. For example, it is well known that in Necturus (Dawson, 1932) and in Cyclostomes (Jordan and Speidel, 1930) granulopoietic foci and masses of lymphoid cells similar to those seen in birds normally occur in the liver. Also, Drinker and Yoffey (1941) conclude that even in those birds possessing lymph nodes, they are poorly developed. Papers on the lymphatics of the chicken published by Baum (1930) Dransfield (1944) and unpublished work done at this Laboratory indicate that the above statement also may be applied to the entire lymphatic system. While vessels can be traced, they appear to be poorly developed, with extremely fragile walls. It appears as though dense connective tissue is almost completely lacking even in the walls of the larger lymphatics. The concept that lymph nodes arise as a collection of undifferentiated mesenchymal cells which subsequently are organized into a lymph node (Miyazaki, 1940) is interesting in this respect. Do chickens, and birds in general, form the original mass of mesenchymal cells with subsequent failure to develop the lymphatic vessels and capsule of a lymph node? Disruption of the connective tissue sheath of the mammalian lymph node has been demonstrated by Furuta (1949), and results in histological changes very similar to the spreading of lymphoid foci of birds. As the incidence of lymphomatosis is high in the Laboratory flock, it is inevitable that any extensive sample of chickens will show various stages of the leukemic process, just as Potter, et al., (1943) were able to study pre-leukemic stages in the mouse. Thus it becomes exceedingly difficult to separate normal from abnormal lymphoid areas, and on the basis of the present data it appears necessary to maintain the hypothesis that at least some ectopic lymphoid areas are normal for birds.

Reviews of the extensive literature on the pituitary-adrenal cortex-lymphocyte relationship have been listed in the introduction. That the expected negative correlation between size of adrenal cortex and lymphoid tissues has not been observed in these data is not surprising, for, within certain limits, size of a gland is not a measure of its physiological activity. Also, while the negative relationship could be expected even if the lymphoid structures were abnormal, hypertrophy of adrenal cortical tissue in response to growth of small lymphoid tumors may have a tendency to make the correlation positive. Never-

the-less, differences both in quantitative histology of the adrenal, growth of the various organs studied, and growth of lymphoid tissues in liver and pancreas indicate that basic physiological mechanisms differ in the various inbred lines. That adrenal hormones are important factors in resistance to tumors has been demonstrated by Diller, et al., (1948); Law (1947); Law and Speirs (1947); Murphy and Sturm (1943, 1944, 1950); Sturm and Murphy (1944). Normal tissues showing a correlation with resistance to tumors have been demonstrated by a study of blood of the rabbit (Casey and Pearce, 1931), number of Peyer's patches in the mouse (Kelsall, 1946), and adrenal-body weight ratios and leucocyte counts in mice (Stein and Cheng, 1948). Correlation of number of Peyer's patches with incidence of mammary carcinoma, reported by Kelsall (1946), is a good corollary of the correlation between percentage of lymphoid tissue in the pancreas and incidence of lymphomatosis in chickens (Lucas and Oakberg, 1950) and present data. From the preceding discussion, it is apparent that correlations based on lymphoid tissues in the pancreas of chickens may represent actual influence of defense mechanisms on tumor tissue, or the relationship may result from a common basis for growth of normal structures and resistance to tumors.

In conclusion, it is apparent that no clear understanding of the function and significance of ectopic lymphoid tissue in chickens can be formulated on the basis of available data. While the present study has failed to establish a relationship with the adrenal cortex, this does not mean that lymphoid tissues of the chicken are not influenced by adrenal hormones. While considerable variance is unexplained, comparison of the four inbred lines used indicates the importance of genetic constitution in growth of the adrenal, visceral organs, and lymphoid tissues in liver and pancreas. It appears certain that a large number of genetic factors and physiological relationships are involved.

Summary

Lymphoid tissues of liver and pancreas have been studied for 158 male and female White Leghorn chickens from four inbred lines.

Growth of ectopic lymphoid foci has been shown to depend upon line, family, and the individual bird. Variations between lines and families suggest genetic differences. Origin of bird differences also may be partly genetic.

Common factors are involved in increase in number and in mean size of lymphoid foci of liver and pancreas. Some relationship in growth of lymphoid tissues in liver and pancreas is indicated, but more data would be necessary to confirm this point.

Amount of lymphoid tissue in liver and pancreas is correlated with pancreas and spleen weights.

Incidence of lymphomatosis is positively correlated with number of lymphoid areas and percentage of lymphoid tissue of the pancreas.

Number of lymphoid areas in the liver gave a positive, significant correlation with ability to survive mortality from all causes.

The data are discussed in relation to pituitary-adrenal cortex-lymphocyte relationships, particularly in reference to lymphomatosis. Evaluation of these data indicates no adequate interpretation of the rôle of ectopic lymphoid foci, but a difference in function of areas in liver and pancreas is suggested. Control of growth of lymphoid areas appears to depend upon the particular group of chickens under consideration, with a large number of variables indicated.

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A COMPARISON BETWEEN MICROTUS DRUMMONDI (AUD. AND BACH.) AND MICROTUS PENNSYLVANICUS PENNSYLVANICUS*

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(Received for publication June 25, 1951)

The genus *Microtus* is one of the most widely distributed of the vole or mouse groups on the North American continent. It appeared on this continent in the Pleistocene epoch according to Scott, but failed to gain a foothold at that time and died out only to re-establish itself in post-glacial times. Its northern limit is well beyond the Arctic Circle. *M. drummondi* has been described by Preble as common in northern Alberta and Saskatchewan and the genus is found throughout the United States, being most plentiful in the temperate regions. Nelson has reported *M. pennsylvanicus* as far south as coastal South Carolina. We found the species *drummondi* in great numbers at Churchill, Manitoba, and north as far as Chesterfield Inlet, Lat. 63 degrees N. The tundra seems well suited to its burrowing habits, while the grasses and low-growing shrubs of the north give it assurance of an abundant food supply. The Ohio species, *pennsylvanicus,* has the same liking for grasses as its northern cousin, but adds grain, vegetables, and fruit to its diet. It shows the same love for low marshy ground. Both forms are of medium size, of compact body, with small eyes and dull body colors. According to Bailey, *pennsylvanicus* is capable of emitting squeaking noises, has a fairly acute sense of smell, and is friendly in disposition, gregarious in habits, a good fighter, sanitary in habits, and polygamous. According to this author, too, its gestation period is about 20 days and 17 hours. He records one female that produced a total of 17 litters in one year. He finds that a single animal may consume more than twice its own body weight in food in one day and estimates the destructiveness of 100 mice at about 2300 pounds of green clover or grass per year. Since a population of 100 to the acre in grass or agricultural regions is not unusual and may reach 1000, the economic loss due to this rodent becomes

*Hereinafter referred to as northern and local species respectively.

TABLE 1

MICROTUS PENNSYLVANICUS VS. MICROTUS DRUMMONDI

Body Weights
Weights in Grams—Differences in Mgms.

No. Name	Body Weight P. E.	Standard Deviation P. E. St. Dev.	C.V.%P. E. C. V.	Differences in milligrams	
37 Loc M	28.05±.8431	7.75±.6070	27.7±2.158	Loc. M. HB M.	2.920±1.050 NS*
61 HB. M	25.13±.6272	7.05±.4451	28.1±1.1713	Loc. F. Hb. F	3.430±1.290 NS
40 Loc F	27.52±.9105	8.60±.6407	29.5±2.225		
35 HB. F	24.09±.9240	10.30±.6581	23.1±1.86		
	Brain Weight				
37 Loc. M	.7501±.006142	.0456 ±.003574	6.07± .4755	Loc M. HB M.	81.1± 6.75 S
61 Hb. M	.6690±.004401	.0510 ±.003126	7.4 ± .4537		
40 Loc. F	.7287±.006137	.0570 ±.004249	7.6 ± .5665	Loc F. HB F.	77.7± 8.12 S
35 Hb. F	.6510±.005331	.04710±.004280	4.71± .6604		
	Thyroid Weight				
37 Loc. M	.004088±.0002192	.00136±.0001792	33.2±2.637	Loc M. HB M.	.685± .241 NS
61 Hb. M	.003403±.000101	.0012 ±.00007217	35.0±2.09		
40 Loc F	.005125±.0002610	.00245±0001821	47.7±3.574	Loc F. HB F.	1.772± .310 S
35 Hb. F	.003353±.000168	.00149±.000121	44.0±3.57		
	Adrenal Weight				
37 Loc M	.00771 ±.0002967	.0241 ±.00188	36.6±2.87	Loc M. HB M.	1.384± .332 S
61 Hb. M	.009094±.0002630	.03320±.00190	36.0±2.07	Loc M. Loc. F.	7.790± .320 NS
40 Loc F	.01550 ±.001215	.01140±.0008566	74.6±5.625	Loc F. HB F.	2.570± 2.130 NS
35 Hb. F	.01807 ±.001753	.01550±.001214	85.0±7.491	Hb. M. HB F.	8.976± .314 S

TABLE 1 (*continued*)

No.	Name	Body Weight	P. E.	Standard Deviation	P. E. St. Dev.	C.V.%	P. E. C. V.	Differences in milligrams		
		Heart Weight								
37	Loc. M	.1976	±.006636	.060	±.00469	30.6	±2.401	Loc M Hb. M.	29.6± 7.85	S
61	Hb. M	.2116	±.004818	.048	±.00296	29.2	±1.753			
40	Loc. F.	.2220	±.008610	.0808	±.006110	35.9	±2.701			
35	Hb. F	.1672	±.007070	.0534	±.004249	31.0	±2.495	Loc F. HB F.	52.6±11.10	S
		Liver Weight								
37	Loc M	1.3615	±.04024	.3625	±.02839	26.6	±2.081	Loc M. HB M	233.5±65.0	S
61	Hb. M	1.1280	±.04128	.480	±.02763	42.0	±2.698	Loc F. HB F	67.5±31.1	NS
40	Loc F	1.5275	±.06072	.5700	±.0428	46.9	±3.561	Loc M. HB F	166.0±72.8	NS
35	Hb. F	1.3600	±.0458	.415	±.0323	30	±2.434	HB M. HB F	232.0±62.5	S
		Kidney Weight								
37	Loc. M	.3389	±.007486	.0670	±.005263	20.0	±1.585	Loc M. HB M	36.8±10.6	S
61	Hb. M	.3631	.00768	.0895	±.005483	24.8	±1.531			
40	Loc F	.3600	±.01011	.1000	±.007486	27.7	±2.084	Loc F. HB F	15.7±20.5	S
35	Hb. F	.3757	±.0175	.148	±.0115	39.0	±3.17			
		Lung Weight								
37	Loc M	.3960	±.01146	.108	±.008094	27.2	±2.050	Loc M. HB M	47.0±16.9	NS
61	Hb. M	.4107	.01214	.147	±.009173	35.0	±3.102			
40	Loc. F	.4025	±.01079	.1029	±.0007756	25.2	±1.922	Loc F. HB F	8.2±18.8	NS
35	Hb F	.3943	.01551	.137	±.01079	35.0	±2.76			

*S and NS: Significant or Not Significant

significant. The annual hay loss is estimated at about $30,000,000.

Because of the great climatic differences between northern Ohio and Churchill, Canada, it was considered worthwhile to make a comparison of the weights of the two closely related species, *Microtus drummondi* and *M. pennsylvanicus*. This comparison included body, brain, thyroid, adrenal, heart, lung, kidney, and liver weights. The northern species was collected during August, 1947, and the Ohio specimens were trapped during September, October, and early November of the same year. Specimens were preserved in 10 per cent formalin and the weights, with the exception of body weights, were taken after preservation. The data were treated statistically. A total of 77 of the local and 96 of the northern species were obtained.

Tables 1, 2, and 3 set forth the weight data of the two groups.

Body Weight

The body weights of the northern species were lower than those of the local species (Table 1). The local males when compared with the northern males showed a difference of 2.92 ± 1.048 grams or 10.4 per cent in their favor, a difference which is not significant. The local exceeded in weight the northern females by 3.43 ± 1.29 grams or 12.1 per cent, again an insignificant difference. It is possible that the particular season (August) in which we collected the northern specimens influenced the body weight since for both the northern males and females, the weight classes from 15 to 20 and from 20 to 25 grams contained the greatest frequencies and over 20 per cent of the northern *Microtus* fell in the lowest weight category. On the other hand it is also possible that we have here an emerging species difference that may have been brought about by environmental factors. The weight values incidentally lie in the same range as Dice found for *Microtus ochrogaster*.

Brain Weight

The brain weights of the local species are actually greater than those of the northern species. So far as the ratio between body weight and brain weight is concerned, the ratios are similar. The ratios $\frac{BW}{Br}$. fluctuate between 1:37.0 and 1:37.9 (Table 2). The local male brain is .0811 grams or 10.8 per cent heavier than that of the northern male brain. The brain of the local females is .0777 grams or 10.6 per

TABLE 2

Name	Surface area in sq. cm.	*S.A.* Brain	*B.W.* Brain	*S.A.* Thyroid	*S.A.* Adrenal	*S.A.* Heart	*S.A.* Liver	*S.A.* Kidney	*S.A.* Lung	Brain k = .94 b =
Local males	83.04	110	37.2	20310	11720	344	63	243	210	.03267
Local females	82.03	112	37.7	16010	5290	369	67.6	227	203	.03230
Northern males	77.21	115	37.9	22700	8400	458	68	212	188	.03382
Northern females	75.08	115	37.0	22380	4150	449	55	199	190	.03270

cent larger than that of the northern females pointing to an apparent significant difference. It will be recalled in this connection that the body weight of the local male is 10.4 per cent and that of the local female is 12.1 per cent in excess of the northern animals.

The local males have the heaviest brain in the entire group. The brain weight difference between local male and female is .0214 ± .00797 grams which may or may not be significant. Taken as they stand, the brain weight differences between the local and the northern males, and those of the local and the northern females appear significant since the weight difference between local male and northern male brain is .0811 grams ± 0.0067 and in the case of the female the brain weight difference is 0.0777 grams ± 0.0081.

When the brain values are plotted against the body weights on double logarithmic grids for the two species (Fig. 1), the slope of

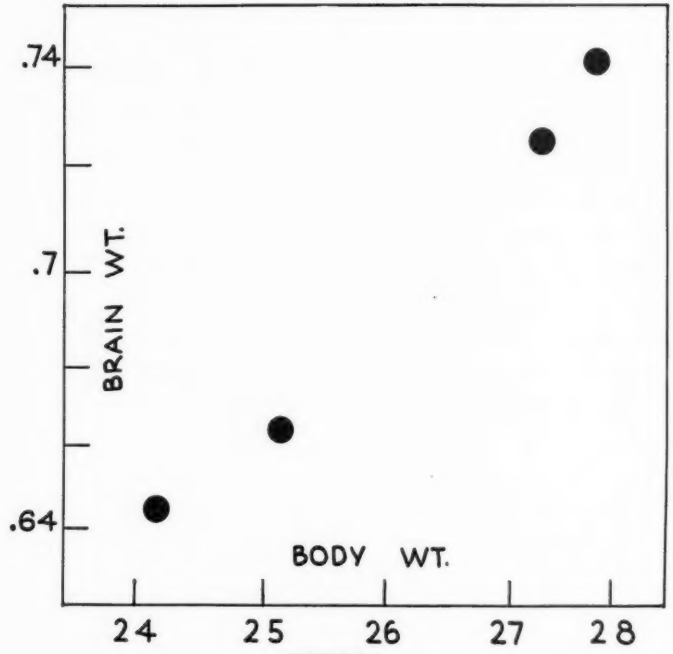


FIGURE 1.
Double logarithmic graph of brain-body weights *Microtus drummondi* and *pennsylvanicus*.

the curve is found to have a value of approximately 0.94, a value which represents almost a direct correlation of brain to body weight, and considerably higher than the value that we have found to hold for vertebrates in general. Calculation of the Snell cephalization coefficient by application of the formula $Y = bx^k$ gives the b values found in Table 2 as calculated for our specimens, and using a k value of 0.94.

With the exception of values observed in the northern males, it will be noted that the b values closely approximate each other. Since it is reasonable to suppose that in the two species the degree of cephalization is the same, these approximations give an indirect clue as to the reliability of the data. Members of a species generally exhibit identical or very closely similar value of b. Between genera greater differences arise, as Hersh showed for titanothere skull dimensions, but even in an order the cephalization values may not be so very far apart.

If the power formula applies there is another way of evaluating relative brain sizes, which probably gives a better insight into fundamental relationships than does a direct comparison. The theoretical brain weight of the 25.13 gram northern males as compared with the 28.05 local males may be calculated by the equation $.7501 \frac{(25.13)}{(28.05)}^{.94} = .6766$ where .7501 represents the average local male brain weight, and the exponent 0.94 the slope of the curve when the Microtus brain is plotted against body weight on double logarithmic grids.

Theoretically, on this basis the northern brain should weigh .6766 grams or .0076 grams more than it actually weighs. By using this difference .0076 as the theoretical difference between the local and northern brains it is less than three times the probable error of difference $\pm$ .0067, indicating that there is no real difference in the brains of the two groups unless our samples fail to show the true conditions. In the case of the female comparison the equation reads: $.7287 \frac{24.09}{27.52}^{.94} = .6430$ the theoretical weight of northern female brain. In other words, the theoretical weight of the northern female brain is .0080 grams less than the actual weight and the difference probably has no real meaning.

On the basis of this evaluation of relative brain size it appears

that there is no significant difference between the brain size of northern as compared with the local microtus species.

Brain body-weight ratios taken by themselves have little meaning since they give but poor clues to the intelligence of the animal. In comparisons between animals of similar body weights, however, the brain size does give an insight into the degree of cephalization and indirectly a clue to the position which the animal occupies in a natural scheme of classification in so far as its neural development is concerned. The brain ratio of the small rodents is high and is absolutely misleading if it is taken to mean that this high ratio implies a high degree of intelligence. We have unpublished data indicating close correlation between brain size and heat production in both cold and warm-blooded animals and it appears that in addition to its other functions we may evaluate brain size in terms of the calories of heat put out by the body, the output correlated with brain size. From this viewpoint the large brain of the rodent is correlated, not with great intelligence, but rather with heat output. Brain size also shows a relation to surface area* but Table 2 indicates that one gram of brain is associated with from 110 to 115 square centimeters of surface area. The individual differences are not significant. The regularity revealed between brain and surface area calls to mind Snell's conception of the validity of this relationship.

Thyroid Weights

The rodent thyroid gland is relatively small. Only the shrew (*Blarina brevicaude*) with an average body weight of 17.60 grams, among the small mammals appears to have a lower thyroid ratio. The ratio of body weight to thyroid in the northern female is 1:6400 (in the shrew, average of 35 animals, the ratio is 7760) while that of the local sparrow is 1:5200, (average of 26 sparrows weighing 23.51 grams) in the human being it is 1:2600. Differences between local and northern males and between the local and northern females emerge. The difference in weight between local and northern male thyroids is .685 mg. $\pm$ .0245, between the females it is 1.772 $\pm$ .031 mg. in favor of the local group in both cases, pointing to a significant difference in the females and possibly in the males. In comparison with these rodent thyroid weights, 102 guinea pigs from our collection with 214.57 grams average body weight and 34.75 mg. thyroid weight,

*Surface areas have been calculated according to the formula: S.A. = k.B.W. 2/3. The k value for the mouse is 9, according to Benedict.

have a body weight which is 6.5 times that of the Microtus and a thyroid weight 8.5 times that of the mouse thyroid. The Microtus thyroid gland in comparison with the adrenal gland is extremely small being about 1/15 to 1/20 in size of the former.

Adrenal Weights

Two striking facts appear even upon superficial examination of the adrenal glands, one is their greater weight when compared with that of the thyroid gland and the other is the weight difference between the male and female adrenal glands. Further, the glands of northern males and females are relatively and actually larger than those of the local species. Thus, in the northern and the local males the difference in gland size is 1.384 mg. $\pm$ 0.394 mg., indicating a significant weight difference in favor of the northern male. The difference between the females is 2.57 $\pm$ 2.13 mg. With the standard error $\pm$ 2.13 mg., no significant difference is indicated. Intra-special comparisons of males and females show significant differences of 7.78 $\pm$ .325 mg. between local males and females, and 9.976 $\pm$ .314 mg. between the northern males and females. This appears to be a case of sex dimorphism, which we have noted also in the adrenal glands of the male and female guinea pig. In a group of 46 male guinea pigs the mean adrenal weight was 134.48 mg. $\pm$ 2.026 and in a group of 56 females the mean adrenal weight was 180.28 $\pm$ 1.809; the difference in the male and female gland weights was 45.80 mg. $\pm$ 2.52. What the meaning of this sex difference is, we cannot state. The medulla of the Microtus adrenal is large compared to its relative size in other orders of mammals. Chance sections of the gland show from 10 to 30 per cent of the area made up of medullary tissue. One might, if speculatively inclined, deduce the nervous habits, the excitable nature, the quick movements, the rapid heart rate, high metabolism, and correlated therewith the relatively short life of these creatures from the size of their adrenal medullae, independent of the evaluation of other systems.

Heart

The heart weight of a mammalian species increases at a lower rate than does the body weight. This can be demonstrated by comparing small with large members of a given species. We find in our studies of the various orders of mammals that it tends to increase from 0.7 to 0.85 times the rate of the body weight increase. This appears logical in view of the work of the heart, for while it keeps the mass

of the body supplied with blood it is also responsible for the maintenance of the blood in the surface vessels and since the surface is relatively smaller in larger bodies, the heart offers a clue to this changing relationship between mass and surface. Klatt (1919) found that the heart weight increase could be expressed by the exponent k in the equation $Y = bx^k$, following the reasoning of Snell and Dubois in their evaluation of brain weights. In numerous tabulations he established a heart k value for mammals of approximately 0.84. Our own averages for Microtus give a value of approximately 0.74 for the genus although individual differences between the two species may run higher (Fig. 2). The mean heart weight for our local males was .1976 ± .0066 grams, that for our northern males was .1680 ± .0048 grams, the weight difference between the two was .0296 grams ±

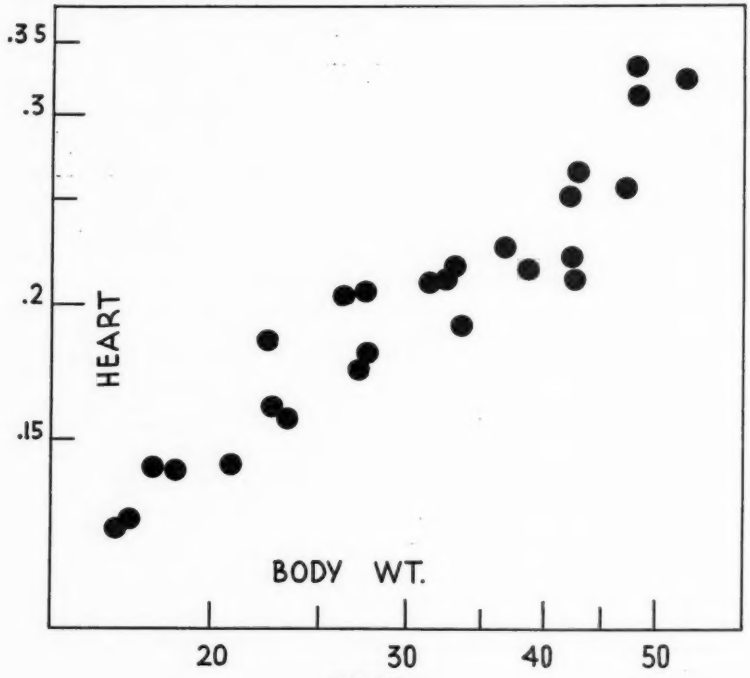


FIGURE 2.

Double logarithmic graph-heart-body weights of *Microtus drummondi* and *Microtus pennsylvanicus*.

.00785 grams, indicating a significant difference favoring the Ohio species. In the females this difference favored the local female by .0526 grams ± .0111 grams again indicating a significant difference.

These established differences may be more apparent than real, however, for if these subordinate parts are thought of as increasing in accordance with the power formula $Y = bx^k$ then the theoretical value of the smaller northern specimens may be calculated as follows: $.2116 \frac{25.13}{28.05}^{.74} = .1758$ grams. The difference between this theoretical weight for the northern male heart and the actual heart weight was .0078 grams. Assuming that the probable error would be of the same order as that indicated for the comparison between the northern and local males, namely ± .0078, on this basis there is no significant difference in weight.

Applying the formula to the local and northern female hearts, the equation reads: $.2220 \frac{24.09}{27.52}^{.74} = .1919$ grams. The actual average heart weight of the northern females was .1672 grams and a difference between the theoretical and the actual heart weight of .0247 grams results with a p.e. of ± .00784. This implies a significant weight difference between the northern and the local female heart that favors the Ohio rodents.

The heart weight is not directly correlated with the surface area (Table 2) for the ratio, $\frac{\text{S.A.}}{\text{Heart}}$ does not result in a constant value as happens when similar calculations are made for the brain. While the values within the species approach each other they do not hold outside the species. As a matter of fact, the brain alone of the systems so far considered shows this close approximation to a constant relationship of brain size to body surface, for both the thyroid and the adrenals show a wide variation when their sizes are compared with the corresponding surface areas (Table 2).

Mean Liver Weights

A statistical evaluation of the liver for this group of rodents shows that it is larger in the female than it is in the male of both species. The difference in liver weight between the local male and the local female is .1660 ± .0728 grams, while between the northern male and female the liver weight difference is .232 grams ± .0625. We

cannot state what the cause of the heavier liver in the lighter female is, nor do we know what the larger liver signifies to the female body economy.

When the liver weights are plotted against the body weights (Fig. 3) for our various weight categories as tabulated in Table 3, an interesting relationship is disclosed.

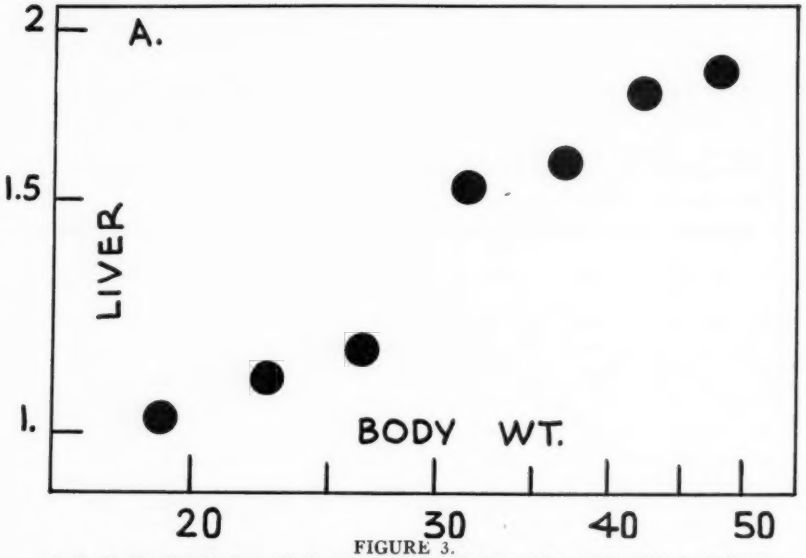


FIGURE 3.

A. B. C. D. Double logarithmic graph of liver-body weight relationship in *Microtus drummondi* and *Microtus pennsylvanicus*. Figures III, A) Local males, B) Local females, C) HB males, D) HB females.

The slopes shown in the graph disclose the fact that in the case of the Hudson Bay female the k value is 1.2 which means that the liver weight advances at a slightly higher rate than does the body weight. In the case of the local female the k value is 1.06 indicating a virtual direct correlation between liver and body weight increase. In the males of both species on the other hand the liver k value for the Hudson Bay males was 0.87 and for the local males the value was 0.81 which accounts for their relatively lower liver weights in the higher body weight brackets. One is tempted to associate the larger liver with the specific demands made on the female body in connection with the bearing as many as 17 litters per year, or with some other specific sexual characteristic.

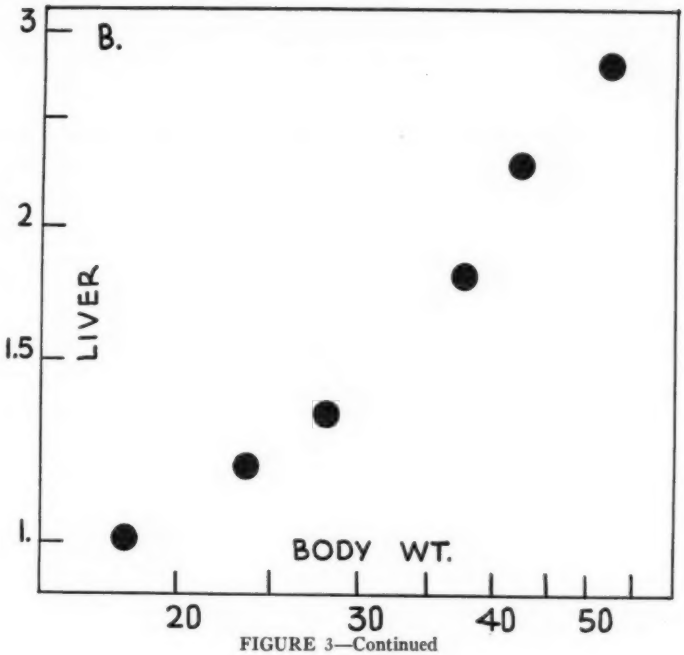


FIGURE 3—Continued

Mean Kidney Weights

The kidney weights ranged from 0.3389 grams in the local male to 0.3757 grams in the northern female. In contrast to the other organs evaluated with exception of the adrenal glands, the kidneys actually weighed more in the lighter northern animals than they did in the heavier Ohio species. The factors which determine this peculiar weight relationship in connection with these glands may possibly be dietetic differences or relative amounts of blood. When the kidneys of the northern and the local males are compared the weight difference is .0242 grams ± .0107 in favor of the northern animal which is hardly significant taken by itself. The northern female kidneys outweigh the local ones by .0157 grams ± .00653 which points to a valid difference.

The kidney weights for the various categories are shown in Table 3.

TABLE 3
WEIGHT BY CLASSES

No.	Body Weight	Brain Weight	Thyroid	Adrenal	Heart	Liver	Kidney	Lung
Local Males—								
5	18.82	.6942	.0028	.0058	.1407	1.0049	.2890	.2993
7	22.52	.7427	.0032	.0092	.1883	1.1229	.2960	.3639
12	26.97	.7545	.0048	.0086	.2035	1.2722	.3103	.3825
6	32.09	.7649	.0041	.0070	.2110	1.5728	.3481	.3585
3	37.10	.7363	.0042	.0065	.2290	1.6620	.3410	.3992
2	42.57	.7789	.0041	.0045	.2203	1.8328	.4128	.5051
2	49.45	.7855	.0040	.0049	.3405	1.9415	.5160	.5600
Local Females—								
5	17.83	.6966	.0031	.0056	.1436	1.0260	.2491	.3112
9	23.29	.7099	.0039	.0083	.1595	1.2041	.3089	.3484
13	27.52	.7296	.0042	.0131	.2062	1.3349	.3349	.4581
3	32.58	.7632	.0076	.0193	.2119	1.7603	.4272	.4606
4	38.40	.7189	.0056	.0251	.2165	1.8554	.4123	.3719
3	43.60	.7178	.0070	.0266	.3695	2.3503	.4547	.5312
3	52.78	.7383	.0091	.0422	.3377	2.8457	.5130	.4946
Hudson Bay Males—								
22	16.83	.6117	.0029	.0054	.1261	0.8440	.3068	.3190
20	22.92	.6737	.0033	.0097	.1604	1.0319	.3459	.4025
8	27.21	.6815	.0035	.0076	.1757	1.1628	.3668	.4156
6	33.64	.6995	.0038	.0105	.1973	1.4239	.4603	.5000
3	41.85	.6846	.0036	.0102	.2516	1.8088	.5059	.7135
2	47.57	.7436	.0056	.0123	.2553	2.4696	.6380	.6788
Hudson Bay Females—								
13	16.84	.6502	.0025	.0103	.1283	0.8909	.3061	.3355
12	21.19	.6560	.0032	.0112	.1435	1.0273	.3142	.3404
1	27.31	.6927	.0025	.0080	.1805	0.9641	.4161	.3348
5	33.24	.6552	.0032	.0403	.2208	1.3738	.4888	.4969
2	42.57	.6889	.0058	.0491	.2618	2.5473	.8606	.7856
2	47.81	.7176	.0060	.0503	.3234	2.7190	.8204	.7335

When these kidney weights are plotted against the body weights (Fig. 4) on double logarithmic grids, the slopes of all the curves are closely similar except that the curves for the local groups starts at a lower level and have a slightly steeper slope (0.697 against 0.625 and 0.67 slope). Based on the actual weights of the local species the calculated kidney weights for our northern group should be 0.3114 for the males and 0.3057 for the females. The actual kidney weights of the northern males are 0.0517 grams, and of the females, they are 0.0700 grams in excess of this weight.

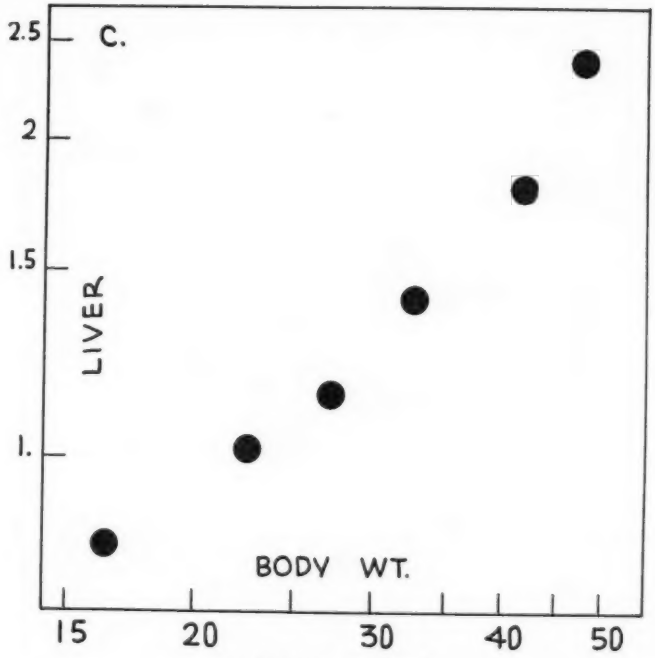


FIGURE 3—Continued

Lung Weights

The lungs of the two species of Microtus show no significant weight difference either when males are compared with males, when females are compared with females, or when intra-special comparisons are made. Plotted against the body weights on double logarithmic grid (Fig. 5), the lung weight increases with definite regularity but at a slower rate than the body increase. This gives a curve with a slope of approximately 0.75, indicating the rate of increase of the lung mass with the body mass.

Lung weight is related to surface area as the quotient $\frac{\text{S.A.}}{\text{Lung}}$ indicates (Table 2). In the Ohio species a smaller amount of lung is correlated per unit area of body surface than in the northern, the intra-special values approaching a constant.

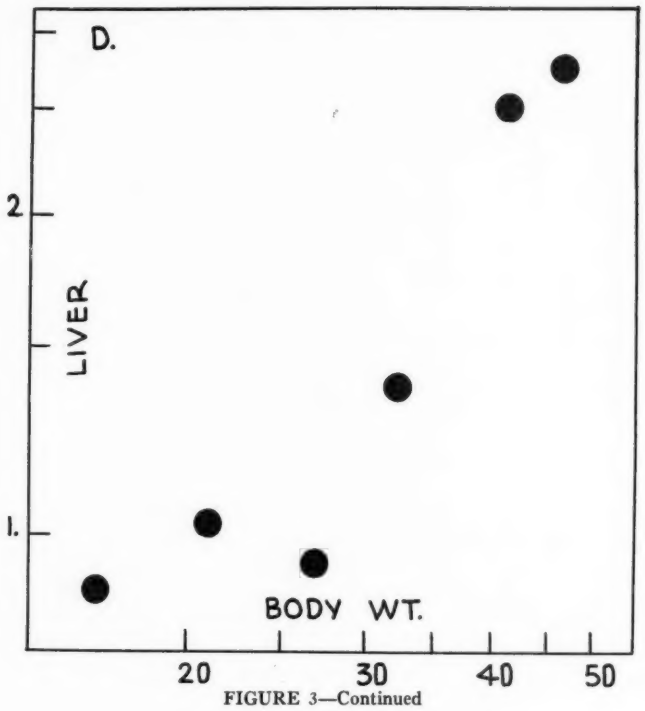


FIGURE 3—Continued

SUMMARY

A comparison of the body, brain, adrenal, thyroid, heart, liver, kidney and lung weights of 75 *Microtus pennsylvanicus* and 96 *Microtus drummondi* indicate the following:

1. The body weights are not significantly larger in the local species (*pennsylvanicus*) as compared with northern (*drummondi*).

2. Brain weights are actually but not relatively heavier in the local species than in the northern one when evaluated according to the equation $Y = bx^k$. $\frac{\text{Calculated Surface Area}}{\text{Actual Brain Weight}}$ tends toward a constant for all classes examined. This constant is 110-115.

3. Thyroid gland weights are relatively small. In the local females the thyroids are significantly larger than in the northern females.

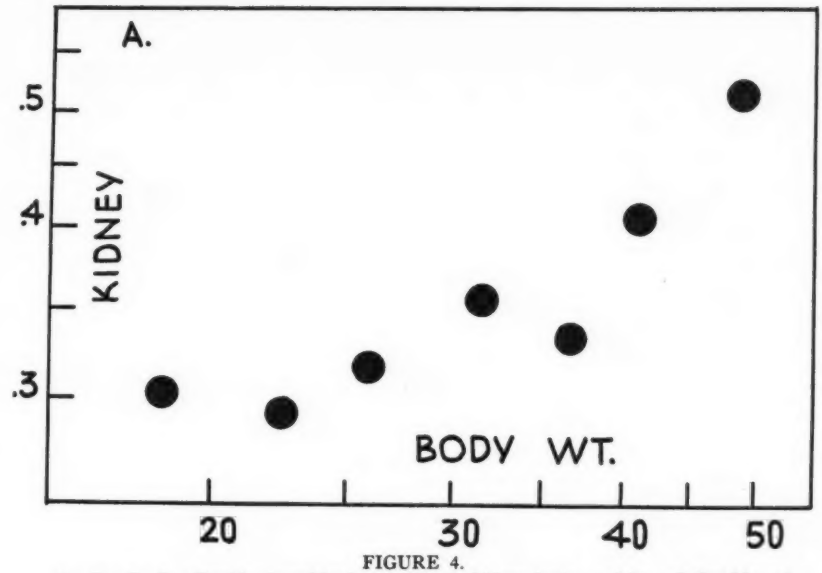
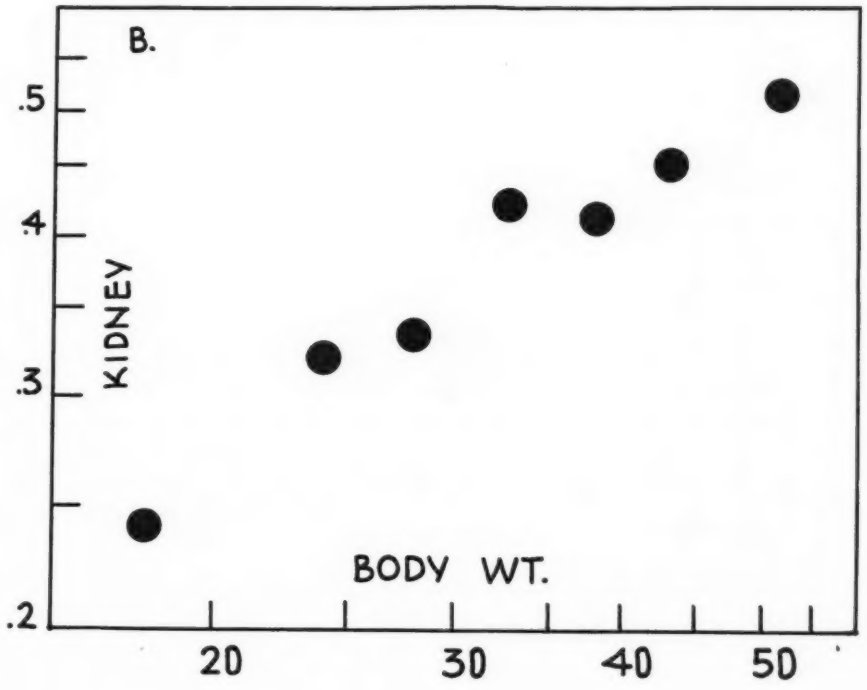
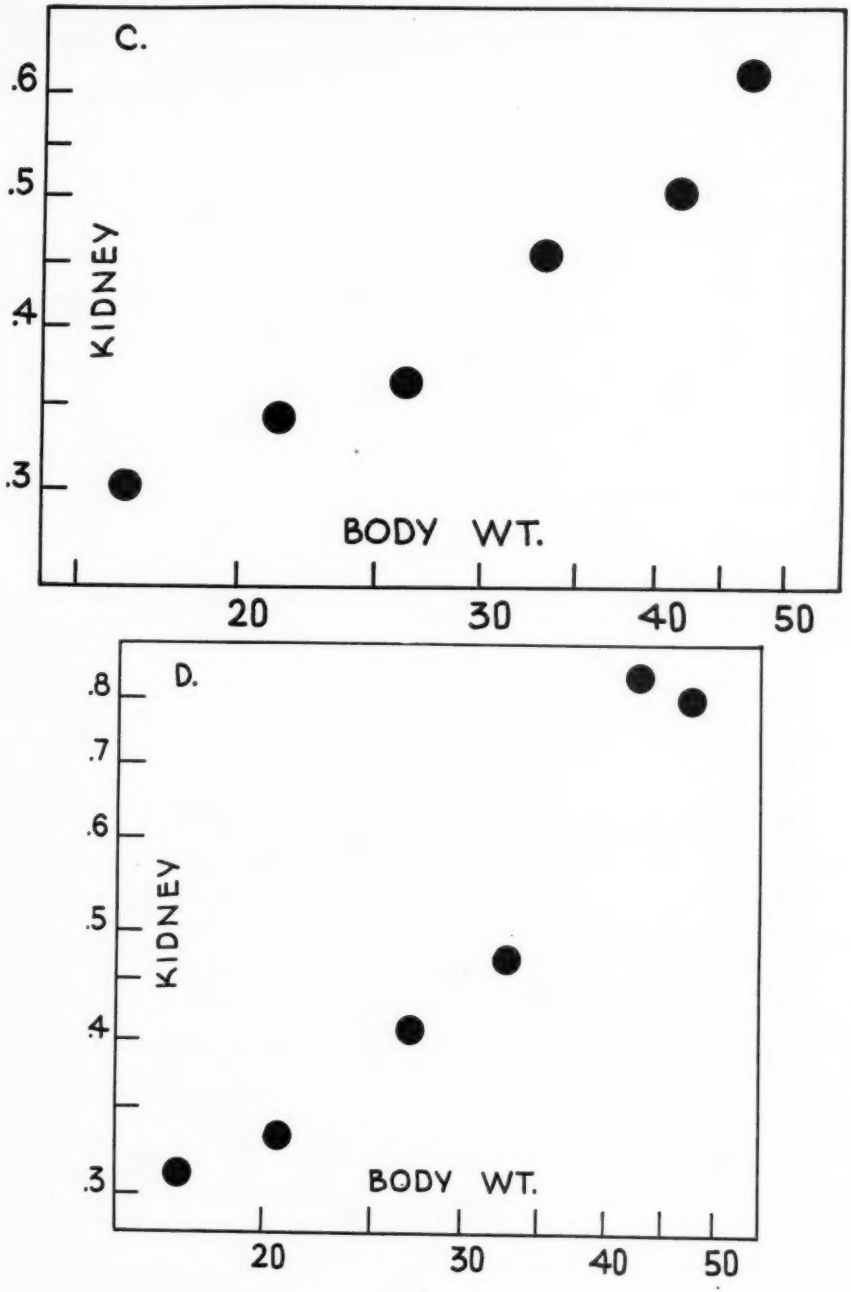


FIGURE 4.

A. B. C. D. Double logarithmic graph of kidney-body weight relationship of *Microtus drummondi* and *Microtus pennsylvanicus*. Figures IV, A) Local males, B) Local females, C) HB males, D) HB females.

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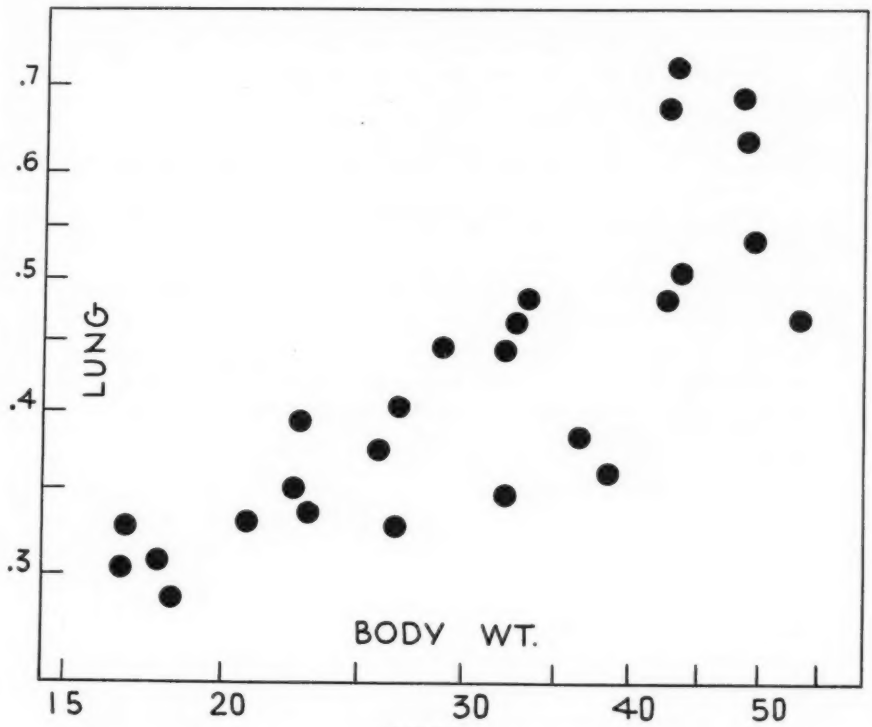

FIGURE 5.
Double logarithmic graph of lung-body weight relationship of *Microtus drummondi* and *Microtus pennsylvanicus*.

4. The adrenal gland weights of the females of both species are significantly heavier than are those of the males of the same species. The adrenal glands of the northern males are significantly larger than those of the local males.

5. The heart weight data indicate that in the local species the hearts are significantly heavier than the northern species.

6. The female liver is heavier than the male liver in each species. Further, it appears significantly heavier in the northern species than in the Ohio species.

7. The kidneys are significantly heavier in the northern females as compared with the local.

8. The lungs of the two species show no significant differences.

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STUDIES IN THE COMPARATIVE ANATOMY OF THE ENDOCRINE SYSTEM

(Thyroid and Adrenal Glands)

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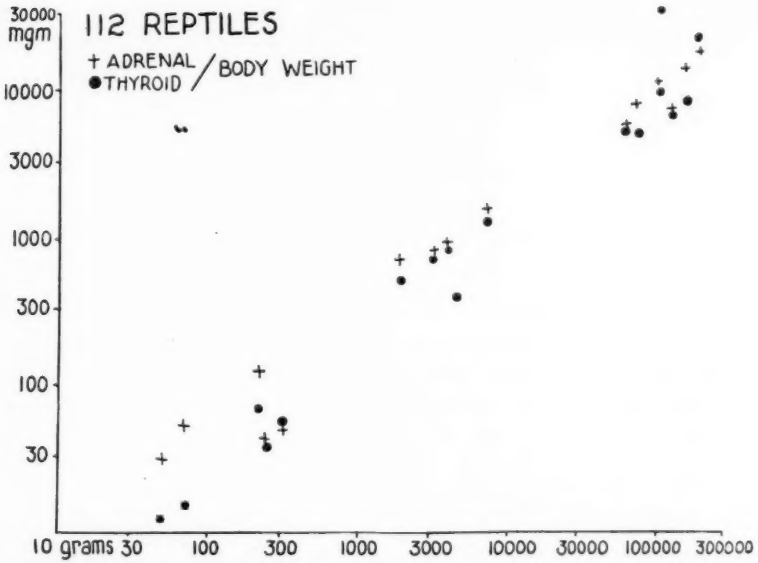
(Received for publication June 25, 1951)

Subordinate parts and systems approach a regular size relationship to the body as a whole within a species. This relationship, which may be expressed by the power formula or the so-called relative growth formula, $Y = bx^k$, has been observed for appendage growth within a species by Huxley[1] and others, for changing skull relationships in Titanotheres by Hersh[3], for endocrine gland size in various species of rabbits by Robb.[2]

The present study represents an application of this equation to thyroid and adrenal relationships to the body as a whole in more embracing categories than species, namely to orders and classes of certain vertebrate groups. The material for the study was gathered in southwestern United States, in Ohio, in Florida, and in Southeast Africa. Ring-dove and domestic fowl data were taken from the literature (Riddle,[4] Mitchell and Juhn,[5] and Landauer and Aberle[6]).

The data plotted on double logarithmic grids exhibit a fundamental regular thyroid and adrenal weight relationship to body weight in spite of the highly divergent types included. The reptile graph (Figure 1) for instance includes representatives of the turtles, lizards, snakes and Crocodilia; the birds include a body weight range from 20 gm. to 123.8 kg. The domestic fowl (Figure 2) shows the greatest deflection from the regularity observed in the weight relationships of these glands. The seeming size depression in these glands apparently represents the effects of domestication since a similar weight depression has been noted by us and others in the relative gland size in the carnivores and ungulates. The same graphic effect is achieved, of course, if the body weight is increased without corresponding gland weight increase. The graphs disclose the striking fact that, in spite of difference due to age, sex, nutritional states,



For the most part the points represent individual values, although in the case of the domestic fowl and the ring-dove they represent averages based upon large numbers of specimens. In our own data, up to 26 specimens are included in some points. Where averages are based upon sufficient samples, they have been subjected to statistical treatment.

FIGURE 1
Reptiles ranging in weight from 50 gm. to 204.8 kg. body weight.

habit and habitat of the animals concerned, a fundamental regularity of weight relationship is maintained within the class of birds and the order Rodentia (Figure 3), with a lesser degree of regularity apparent in the primates. Among the large primates (Figure 4) the human thyroid (average of 3, 23.23 gm.; normal 21-27 gm.) is relatively from 2 to 8 times as large as that in the chimpanzee and gorilla.

Conclusions. A study of the thyroid and adrenal weight relationships in a large series of vertebrates indicates that the weight of these glands tends to approach a constant ratio within a given order as well as in a class. This ratio may be expressed by the so-called relative growth formula, $Y = bx^k$.

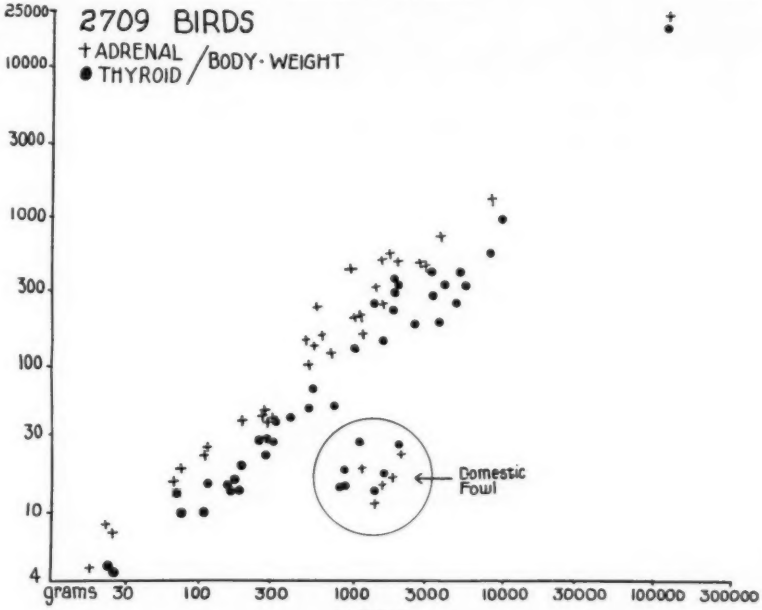


FIGURE 2
A study of 2709 birds including data from the literature 4, 5, 6.

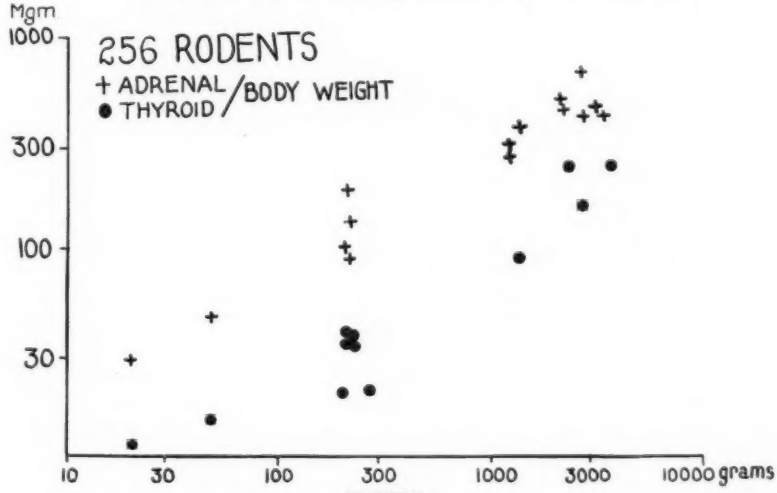


FIGURE 3
The outstanding characteristic of rodents is the great difference between adrenal and thyroid size

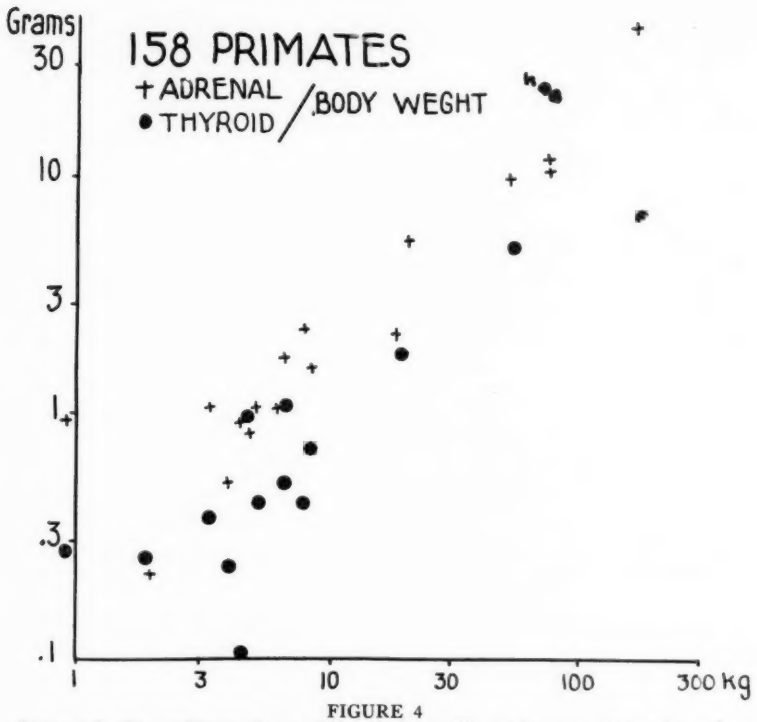


FIGURE 4
New and old world monkeys, chimpanzees, gorilla and man. Note the unique position of human thyroid marked h on graph.

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QUANTITATIVE GROWTH IN OBELIA COLONIES IN CULTURE: II. SEASON CHANGES IN RATES OF BASIC ACTIVITIES

B. Proliferation

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"All perfection in this life has some imperfection clinging to it, and no speculation is without a certain obscurity."

St. Thomas à Kempis: Imitatio Christi

(Received for publication June 27, 1951)

Introduction

Growth of Obelia hydranths and gonangia is defined by successive dominance of initiation, proliferation, differentiation, and organization, with constructive substance increase taking place during the progression. Rates change as season advances (April-September). The generally applicable factors thereof, plus procedures, are given in the 1936; 1942; 1943; 1945; 1946; 1950; 1951 reports.

Proliferation is cell increase in number. Its mark here is expansion of bud to inverted cone; its measure (rate) the percentage of buds which go on to later stages during a 24-hour time-slice. It has 14 sources (expressions), 6 hydranth, 8 gonangial. The hydranth are: initially present (IP) buds, buds coming into being during culture from hydrocaulal anlagen, IP empty hydrothecae, IP broken pedicels, empties from IP seniles, and empties from seniles from IP complete hydranths. Gonangial has 3 sources present at beginning of culture, viz. IP buds, IP minus-quarters (-¼); and IP quarters (¼). And 5 which come into being during culture, viz. buds from hydrocaulal anlagen, minus-quarters from IP buds, quarters from IP minus-quarters, minus-quarters from buds from hydrocaulal anlagen, and quarters from minus-quarters from IP buds (Cf. Hammett, 1943: 1950).

All are exposed to the same season changes in environment. Thus season change in exogenous factors is the same for all, and the difference between expressions in course of rate is grounded in endogenous, not in exogenous factors.

The Data

Rate of each expression in each period (week) over the months from April to September in each year is in Table 46: that for all years combined in Table 48 of the 1950 report. Charting showed each has distinctive trend in most years. So rate of each in all years

TABLE 1
CORRELATION BETWEEN RATE AND TEMPERATURE: PROLIFERATION HYDRANTHS

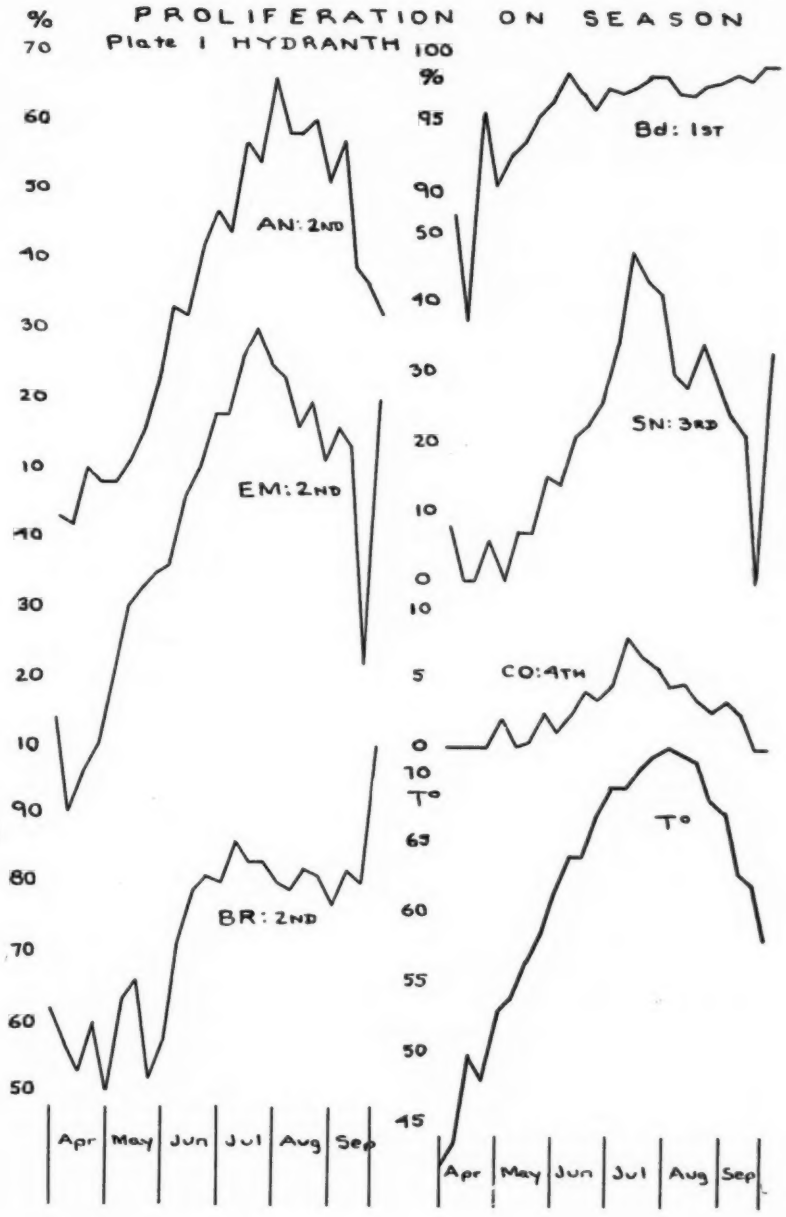
By Month

	Bd (1)						AN (2)						EM (2)					
	Periods			Years			Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
AP	3	1	0	2	0	0	2	2	0	1	1	0	1	2	1	1	1	0
MY	11	3	6	4	0	3	13	3	4	5	0	2	9	6	5	4	1	2
JN	7	9	12	3	3	1	17	7	4	5	0	2	15	8	5	5	2	0
JY	8	7	13	3	2	2	17	5	6	5	0	2	13	10	5	3	2	2
AG	4	8	16	2	3	2	12	7	9	4	1	2	8	12	8	2	4	1
SP	5	3	8	3	1	3	12	2	2	5	1	1	6	8	2	1	3	3
Tot	*38*	*31*	*55*	*17*	*9*	*11*	*73*	*26*	*25*	*25*	*3*	*9*	*52*	*46*	*26*	*16*	*13*	*8*
	BR (2)						SN (3)						CO (4)					
AP	1	3	0	0	2	0	0	3	1	0	2	0	0	0	4	0	0	2
MY	9	6	5	3	1	3	6	8	6	2	3	2	5	2	13	3	1	3
JN	11	12	5	3	2	2	19	3	6	6	1	0	14	8	6	4	1	2
JY	9	12	7	2	4	1	14	8	6	4	1	2	14	9	5	4	3	0
AG	9	9	10	2	4	1	10	10	8	3	3	1	11	9	8	4	3	0
SP	7	6	3	2	1	4	8	5	3	3	2	2	5	8	3	1	3	3
Tot	*46*	*48*	*30*	*12*	*14*	*11*	*57*	*37*	*30*	*18*	*12*	*7*	*49*	*36*	*39*	*16*	*11*	*10*

By Year

	Bd (1)						AN (2)						EM (2)					
	Periods			Months			Periods			Months			Periods			Months		
33	5	2	11	2	0	3	11	4	3	4	0	1	10	5	3	3	1	1
34	6	2	7	4	0	1	9	3	3	4	0	1	9	4	2	3	0	2
35	4	5	8	2	2	1	10	2	5	3	0	2	7	4	6	2	1	2
36	3	1	13	2	0	3	7	6	4	2	2	1	6	7	4	2	2	1
37	3	9	5	1	4	0	11	1	5	4	0	1	4	8	5	1	4	0
38	8	5	6	3	1	2	13	3	3	5	0	1	10	5	4	4	1	1
39	9	7	5	3	2	1	12	7	2	3	1	2	6	13	2	1	4	1
Tot	*38*	*31*	*35*	*17*	*9*	*11*	*73*	*26*	*25*	*25*	*3*	*9*	*52*	*46*	*26*	*16*	*13*	*8*
	BR (2)						SN (3)						CO (4)					
33	6	7	5	1	2	2	11	3	4	3	0	2	8	4	6	2	0	3
34	4	7	4	2	3	0	7	5	3	1	2	2	4	8	3	0	3	2
35	8	3	6	3	0	2	10	1	6	4	0	1	7	4	6	2	1	2
36	7	6	4	3	1	1	7	5	5	3	2	0	6	5	6	3	2	0
37	5	7	5	1	3	1	6	6	5	2	2	1	6	6	5	3	2	0
38	7	8	4	1	3	2	7	9	3	2	3	1	11	4	4	4	1	1
39	9	10	2	1	2	3	9	8	4	3	3	0	7	5	9	2	2	2
Tot	*46*	*48*	*30*	*12*	*14*	*11*	*57*	*37*	*30*	*18*	*12*	*7*	*49*	*36*	*39*	*16*	*11*	*10*

S — Same direction of change: O — Opposite direction of change: N — No comparison


PROLIFERATION ON SEASON
Plate I HYDRANTH
%
AN: 2ND
EM: 2ND
BR: 2ND
Bd: 1ST
SN: 3RD
CO: 4TH
T°
Apr
May
Jun
Jul
Aug
Sep

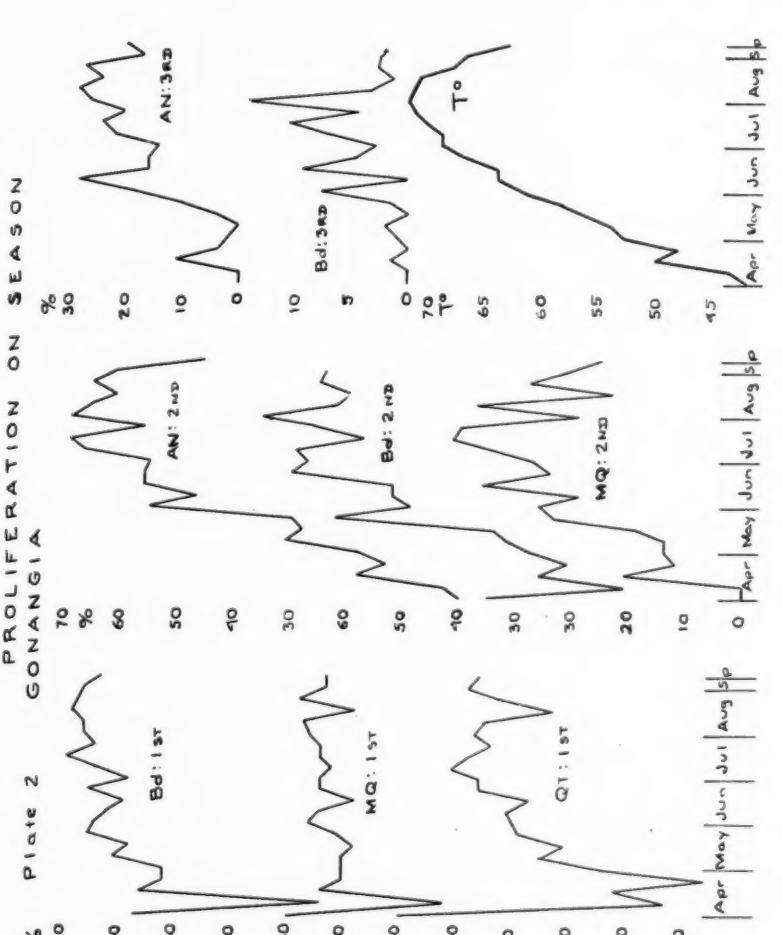

PROLIFERATION ON SEASON
GONANGIA
Plate 2
%
Bd: 1ST
MQ: 1ST
QT: 1ST
AN: 2ND
Bd: 2ND
MQ: 2ND
AN: 3RD
Bd: 3RD
T°
Apr
May
Jun
Jul
Aug
Sp

TABLE 2

CORRELATION BETWEEN RATE AND TEMPERATURE CHANGE: PROLIFERATION GONANGIA

BY MONTH

	Bd (1)						MQ (1)						QT (1)						AN (2)					
	Periods			Years			Periods			Years			Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
AP	3	1	0	2	0	0	2	2	0	1	1	0	3	1	0	2	0	0	4	0	0	2	0	0
MY	7	4	7	2	1	3	4	5	9	1	2	3	7	6	5	3	2	1	7	5	6	3	2	1
JN	8	6	10	3	2	1	9	7	8	3	3	0	13	6	5	5	1	0	9	8	7	1	2	3
JY	4	12	8	1	5	0	4	11	9	1	4	1	6	13	5	1	4	1	8	8	8	2	2	2
AG	4	9	11	0	2	4	3	8	13	1	2	3	7	9	8	2	3	1	7	10	7	2	2	2
SP	4	3	5	1	1	4	4	3	5	3	2	1	5	3	4	2	1	3	7	4	1	3	1	2
Tot	*30*	*35*	*41*	*9*	*11*	*12*	*26*	*36*	*44*	*10*	*14*	*8*	*41*	*38*	*27*	*15*	*11*	*6*	*42*	*35*	*29*	*13*	*9*	*10*
	Bd (2)						MQ (2)						AN (3)						Bd (3)					
AP	2	2	0	1	1	0	2	1	1	1	0	1	3	0	1	2	0	0	1	0	3	1	0	1
MY	9	3	6	4	1	1	7	6	5	1	2	3	5	5	8	3	2	1	3	3	12	2	2	2
JN	8	11	5	1	3	2	12	8	4	4	1	1	12	6	6	4	2	0	5	1	18	3	0	3
JY	8	11	5	3	3	0	10	10	4	3	3	0	10	6	8	4	2	0	4	10	10	0	4	2
AG	8	9	7	2	3	1	6	12	6	2	3	1	5	10	9	2	4	0	6	6	12	3	2	1
SP	4	6	2	0	2	4	9	1	2	5	0	1	6	5	1	2	1	3	3	2	7	1	0	5
Tot	*39*	*42*	*25*	*11*	*13*	*8*	*46*	*38*	*22*	*16*	*9*	*7*	*41*	*32*	*33*	*17*	*11*	*4*	*22*	*22*	*62*	*10*	*8*	*14*

TABLE 2 (*continued*)

BY YEAR

	Bd (1)						MQ (1)						QT (1)						AN (2)					
	Periods			Months			Periods			Months			Periods			Months			Periods			Months		
34	0	2	13	0	1	4	2	3	10	0	1	4	4	5	6	3	1	1	8	2	5	4	0	1
35	3	6	8	1	2	2	3	3	11	2	1	2	6	6	5	1	2	2	6	6	5	1	1	3
36	3	5	9	1	3	1	3	3	11	2	2	1	6	5	6	2	2	1	6	6	5	1	1	3
37	5	6	6	1	2	2	5	8	4	1	3	1	6	7	4	2	2	1	5	6	6	2	2	1
38	10	5	4	4	1	1	6	8	5	2	4	0	8	7	4	4	2	0	8	7	4	3	3	0
39	9	11	1	2	2	2	7	11	3	3	3	0	11	8	2	3	2	1	9	8	4	2	2	2
Tot	*30*	*35*	*41*	*9*	*11*	*12*	*26*	*36*	*44*	*10*	*14*	*8*	*41*	*38*	*27*	*15*	*11*	*6*	*42*	*35*	*29*	*13*	*9*	*10*
	Bd (2)						MQ (2)						AN (3)						Bd (3)					
34	4	9	2	0	3	2	5	8	2	1	3	1	8	4	3	3	1	1	5	5	5	2	2	1
35	7	3	7	3	1	1	7	4	6	3	1	1	5	5	7	3	1	1	2	1	14	1	1	3
36	9	4	4	3	2	0	9	4	4	4	1	0	4	6	7	2	3	0	2	5	10	1	3	1
37	7	5	5	2	0	3	7	6	4	3	1	1	4	7	6	2	3	0	3	5	9	1	1	3
38	6	9	4	2	3	1	8	8	3	3	2	1	9	3	7	3	1	2	6	3	10	3	0	3
39	6	12	3	1	4	1	10	8	3	2	1	3	11	7	3	4	2	0	4	3	14	2	1	3
Tot	*39*	*42*	*25*	*11*	*13*	*8*	*46*	*38*	*22*	*16*	*9*	*7*	*41*	*32*	*33*	*17*	*11*	*4*	*22*	*22*	*62*	*10*	*8*	*14*

S — Same direction of change: O — Opposite direction of change: N — No comparison

combined is used: Plate 1 for hydranth; Plate 2 for gonangial. Also charted is the 7-year average temperature over the same weeks as gotten from Table 13 of the 1945 study. Extent of period-to-period rate-temperature correlation is given in Table 1 for hydranth; Table 2 for gonangial. Variability by expression, month, and year is in Table 3.

TABLE 3

VARIABILITY IN EXPRESSION OF PROLIFERATION (AD/M) 100

	By Month					
	April	May	June	July	Aug.	Sept.
Source			*Hydranths*			
N	*6*	*25*	*28*	*28*	*28*	*16*
Bd:1st	5.3	2.8	2.0	1.9	2.9	1.5
AN:2nd	64.1	48.5	25.6	21.8	15.1	22.7
EM:2nd	84.5	43.1	20.4	13.5	19.7	26.7
BR:2nd	15.8	28.6	12.6	9.4	17.1	8.3
SN:3rd	100.0	86.1	39.8	23.6	38.5	40.9
CO:4th	Indt.	131.1	74.4	36.5	56.6	55.1
			Gonangia			
N	*6*	*22*	*24*	*24*	*24*	*12*
BD:1st	9.6	7.1	7.7	5.2	4.1	4.2
MQ:1st	7.2	7.3	6.7	7.3	6.0	5.0
QT:1st	23.3	12.0	11.2	8.6	16.7	9.8
AN:2nd	58.3	32.2	33.6	25.1	19.2	27.4
BD:2nd	24.9	29.2	26.6	18.9	24.0	18.8
MQ:2nd	95.3	52.9	47.8	28.6	47.7	59.5
AN:3rd	100.0	120.0	72.6	40.1	49.8	62.7
Bd:3rd	166.7	154.8	149.9	101.0	121.7	150.0

	By Year (Season)						
	1933	1934	1935	1936	1937	1938	1939
			Hydranths				
N	*19*	*16*	*18*	*18*	*18*	*20*	*22*
Bd:1st	1.9	1.0	1.8	1.2	2.4	2.2	2.8
AN:2nd	37.2	26.3	40.2	41.7	36.0	50.9	48.3
EM:2nd	34.4	26.8	25.6	31.9	18.8	40.7	44.0
BR:2nd	20.5	10.2	20.6	9.5	10.4	16.0	17.2
SN:3rd	52.7	50.6	54.8	53.4	39.6	74.5	64.9
CO:4th	67.4	59.6	90.0	75.9	51.5	77.3	55.0
			Gonangia				
Bd:1st	—	0.4	3.3	3.1	5.9	5.9	12.8
MQ:1st	—	1.3	2.9	2.6	6.3	6.4	10.2
QT:1st	—	3.9	6.2	11.4	22.6	11.7	14.5
AN:2nd	—	17.9	28.7	28.2	28.8	41.0	55.0
Bd:2nd	—	17.6	23.2	19.7	35.5	32.0	35.8
MQ:2nd	—	22.6	40.6	44.7	48.5	29.5	62.2
AN:3rd	—	41.4	58.8	89.7	73.6	70.7	90.1
Bd:3rd	—	92.1	170.0	146.2	103.0	114.3	143.8

Timing of rate change on season may differ in different years, because of year to year difference in emergence of colony growth from winter inhibition, and in timing of season change in exogenous factors. Such, however, is no bar to interpretation, for the composite trend is that usually found in the years separately. Thus—*in hydranths*: those of AN-1st, EM-2nd, SN-3rd, CO-4th are well shown; and that of BR-2nd is indicated; in all 7 years. That of Bd-1st is well shown in 6. *In gonangia*: that of AN-2nd is well shown in all 6 years. Those of MQ-2nd and AN-3rd in 5. Those of QT-1st, Bd-2nd, and Bd-3rd in 4. Rate of proliferation from IP buds (Bd-1st) and IP minus-quarters (MQ-1st) is frequently 100 per cent or close thereto in 1934, 1935, and 1936. So no season trend is determinable. But rate in 1937, 1938 and 1939 is less. And course of change is evident and the same in each. It is epitomized in the 6-year composites, which are therefore taken as trends of season change in these expressions.

Analysis and Comment

Two types of overall trend occur. 1. Rate starts high; immediately drops sharply to low; rises sharply, then slowly; and tapers off to virtual asymptoticism (*Hyd.* Bd-1st: *Gon.* Bd-1st, MQ-1st, QT-1st). 2. Rate increases to peak and then decreases as season advances (*Hyd.* AN-2nd, EM-2nd, BR-2nd, SN-3rd, CO-4th: *Gon.* AN-2nd, Bd-2nd, MQ-2nd, AN-3rd, Bd-3rd).

1. This course characterizes proliferation from sources present at beginning of culture (1st order), or that which occurs early in culture. Presumably before colony weakening by culture conditions is significant (Hammett, 1950: 1951). It may be interpreted as product of successive dominance of three different factors.

Temperature is season-low when rate is high at start. So this is not the factor. Colonies at beginning season are very young. Early cell increase in number is indomitable. This is why rate is high in 1st period April, despite season low in temperature. It is endogenously, not exogenously (Season) determined.

Low rate after initial high occurs when temperature is still very low. Subsequent rate increase coincides with sharply rising temperature. Up to early June when rate tends to level off. Period-to-period changes in rates during this time tend to positive correlation with those of temperature (Tables 1, 2). Such is present in each expression. And positive in 19; negative in 7; with none in 6 of 32 comparisons

(4 expressions x 8 progressions: from 1st April to 1st June period). The initial drop and subsequent rise in rate is temperature-induced; is exogenously, not endogenously factored.

Course of rate from early June is not that of temperature. Strong rise and decline is absent. And period-to period changes in rates tend to negative—not positive—correlation with those of temperature. Such is found in each expression. And negative in 28; positive in 12; with none in 14 of 54 comparisons (3 gonangial expressions x 13 progressions: 1 hydranth x 15: from 2nd June to 4th Sept. period). Thus temperature is not the determinant of the levelling off phase (Cf. 1951 report).

This phase is like and coincidental with that of population growth. It occurs about the time colony activity comes to steady state—both of which events are products of colony maturing. Furthermore, the negative correlation with temperature is like that of population growth, which stems from maintenance. And it is in maturity that maintenance activity supersedes that of growth (1945; 1950; 1951 reports). The assumption is that colony maturing is responsible for the levelling off phase—that it is endogenously, not exogenously, determined.

Thus the course of 1st order proliferation rate on season has three phases which result from the successive dominance of three different factors. Viz. A beginning high due to colony youngness: an intermediate decline and subsequent rise due to temperature; and a levelling off due to colony maturing.

Record of 1st order proliferation in hydranths is had for 24 periods: in gonangia for 22. Circa 34 per cent is exogenously conditioned (8/24 hyd: 8/22 gon): 66 per cent endogenously. No such dichotomy and no such temperature intrusion is overt in 1st order initiation. There each 1st order course is dominated by the single endogenous factor of colony maturing (1951). Why the difference? Why the dichotomy?

Processes of cell division differ from those of shift in cell activity from no-growth to growth (initiation). The difference is endogenous. It determines the difference in rate and course of rate on season (1936; 1942; 1950). Exogenous factors are same for both. For both are expressed in virtual simultaneity in the same milieu in the same 24-hour time-slices in the same weeks, months, and years. Difference in sensitivity of proliferation and initiation to season change in temperature is clearly due to the endogenously determined difference in nature of the subtending chemical processes.

The dichotomy is due to temperature. a. Why is temperature effective? b. Why is it not effective over the entire course? c. Why is it effective during the 2nd April to 1st June periods? while answers are guesses such focus attention on problems needing solution.

a. Four possibilities: 1. Positive rate-temperature correlation may be spurious. ii. Culture conditions. iii. Constructive substance increase may be the responsive activity. iv. Proliferation processes may be particularly sensitive to temperature change.

i. *For spuriousness*: If rate change is dominated by temperature, such would be expected over the whole course on season. As found in 2nd and 3rd order initiation (1951). But here such happens only during part of the course. *Against*: The fact cell division is product of subtending chemical reactions. The fact rate of such generally increases and decreases with rise and fall in temperature. And the fact positive correlation between the two are additive evidence rate change is due to temperature change. Spuriousness is doubtful.

ii. *For culture conditions*: Is the fact rate is lower during some periods of temperature affect (2nd April-2nd May) than when no affect is overt (June 2nd-Sept. 4). For lower rate can mean delay in appearance of activity in culture. To a time when colonies are weakened. And opened to reaction to season change in temperature (1950; 1951). *Against*: Is the fact lower rates are still high-bracket (86-90% of potentiality). Hence activity is presumably expressed early in culture. Before colony reaction thereto is important. And the fact rates in later periods of temperature affect (3rd May to 1st June) range the same as where course is not temperature conditioned (2nd June to 4th Sept.). Which is presumptive evidence time of appearance ergo culture conditions are essentially the same in both phases. Culture affect is doubtful.

iii. Cell increase in number dominates bud expansion to cone. But cell specialization, segregation, and increase in substance are not entirely absent. And cell maintenance is associated with all (1943; 1950). Temperature affect might stem from reaction of some other activity than proliferation. For activities are interdependent (1946). But initiation, differentiation, organization and maintenance are out. No evidence is had their 1st order expressions are positively correlated with temperature during the time such is the case with proliferation.

No rate-temperature data are had for substance increase. But most of the size increase of hydranths occurs during proliferation dominance. And such is considerable in gonangia (1943; 1950).

Impression is that colony size increase is major during time of temperature direction of proliferation course (2nd period April to 1st June). It is probable that growth in mass is more sensitive to exogenous than endogenous factors. Thus increase and decrease in growth in substance occasioned by rise and fall in temperature during time of active proliferation could result in increase and decrease in rate thereof. Hence the idea substance increase rather than proliferation may be the primary reactant.

Against this is fact substance increase may occur without cell increase in number. The fact cell increase in number may occur without increase in substance. The probability change from spheroid to cone is product of differential cell multiplication: not of substance increase. The probability that here as elsewhere, cell division provides foundation for cell increase in size. Thus, size increase as bud changes to cone is sequel not basis of proliferation dominance. And the fact proliferation processes are chemical reactions. The rate whereof is positively correlated with temperature change. Constructive substance increase is out as primary. With reservation it may participate.

iv. The possibility proliferation processes are not particularly sensitive to temperature comes from the fact such is absent in 1st period April and from 2nd June on. The possibility they may be comes from the fact proliferation is the only activity whose 1st order rates are temperature directed over the periods from 2nd April to 1st June. The guess is the temperature affect is real and due to a particular sensitivity thereto of processes peculiar to proliferation.

b. Rate differences between different activities (prol. vs. init.): between expressions of same activity in different structures (hyd. vs. gon.): and between expressions of same activity of different function (pop. growth vs. pop. main) are endogenously set. They hold regardless of season change in environment. Now circa 66 per cent of the proliferation rate course on season is endogenously determined. Endogenous control is primary (1950). Thus temperature is not effective over the entire course because endogenous factors are dominant. In other words, the particular sensitivity of proliferation processes to temperature succumbs to the more basic control of rate exerted by endogenous processes of colony development-as-a-whole.

c. Most of colony progress to maturity: most of its growing up occurs from 2nd period April to 1st June. Organismic sensitivity to outside forces is generally greatest during the time of most active growingupness. The time of adjustment learning. Before pattern is

fixed by increasing entropy of advancing maturity. This may be why temperature is effective over these periods.

The dichotomy of course into temperature and endogenously directed phases may thus be due to heightened sensitivity of organism to outside influences during time of most active growingupness plus special sensitivity of proliferation processes to temperature change. This completes analysis of season change in rate of 1st order proliferation which occurs before colony weakening by culture conditions is important. Now for proliferation from sources arising during culture. Or activity expressed in colonies somewhat weakened by culture conditions. Here—

2. Rate increases strongly as season advances; then falls away. Such is marked in 8 of the 10 expressions (*Hyd*:An-and; EM-2nd; SN-3rd; Co-4th. *Gon*: AN-2nd; MQ-2nd; AN-3rd; Bd-3rd): shadowed in 2 (*Hyd*:Br-2nd. *Gon*: Bd-2nd). The course is like that of temperature (T°). And trend to positive correlation therewith dominates the period-to-period changes (Tables 1,2). While this suggests the course-as-a-whole is temperature-directed: there are variants. And 1st order data show temperature conditioning may be partial. So particulate analysis is needed. Frequency of superiority of positive correlation over negative will be used. Base procedure is in the 1945; 1950; 1951 reports.

The course of 1st order expressions is temperature-directed during the time of temperature rise: not during its decline. So rate-temperature correlations will here be examined separately on this division. Viz. According to rising rate and temperature. And falling rate and temperature.

During time of rise: positive relation is superior in 101 hydranth items: negative in 27. Is superior in 63 gonangial, in favor in 27. Positive holds in 4 of 5 hydranth expressions: in 3 of gonangial: negative in none. The course of rate change on season is temperature directed during time of rising rate and temperature.

During time of decline: overt trend to positive superiority is feeble. But (a) such is more frequent than in the asymptotic phase of 1st order course: and (b) distinct trend emerges when the disturbing effect of high temperature is eliminated.

a. In hydranths 1st order expression; positive correlation superiority is 50 per cent of negative (6PS/12NS). While in 2nd, 3rd, 4th orders it is 124 per cent (41PS/33NS). In gonangia 1st order positive superiority is 30 per cent of negative (7PS/23NS). While in 2nd and

3rd it is 67 per cent (30PS/45NS). Positive correlation occurs more than twice as often in proliferation from sources arising during culture. Join this with fact these rates decline as temperature falls, while those from IP sources do not. And it is evident the post-rise course of the former is more factored by temperature than the latter.

b. Start of rate decline precedes that of temperature by two weeks (4th period July on average vs. 2nd Aug: Plates 1, 2,). It coincides with arrival of temperature at its narrow maximum range. Such high may be harmful to colonies. For these tend to go to pieces on days of unusual heat. If the three critical periods be deleted it is found that: positive correlation is superior in 35 hydranths cases, negative in 23. Positive is superior in 26 gonangial, negative in 25. Positive holds in 2 of 5 hydranth expressions, negative in 1. And in 4 of 5 gonangial. Thus positive correlation of rate with temperature change occurs during time of rate decline as well as during its time of rise. The conclusion is that the course is temperature directed.

This is attributable to the fact in-culture appearance of 2nd, 3rd, 4th order proliferation occurs as colonies are being progressively somewhat weakened by culture conditions. And hence more sensitive to exogenous factors (1950; 1951).

In general the lower the rate the later the appearance of activity in culture. The later the appearance the greater the sensitivity. Because of increase in colony weakening. Overall rates of 2nd order activity in hydranths are: An-36.1, EM-53.3, BR-75.3. In gonangia: MQ-31.2, AN-46.1, Bd-49.5 (Table 48: 1950). Compare these seriations with conformity of rate course to that of temperature (Plates 1, 2). The lower the rate the better the agreement. Compare them with the rate-temperature correlations (Tables 1, 2). The lower the rate the more frequent the positive associations. Temperature factoring increases as rate decreases. Or as delay in expression of activity and colony weakening increases. The concatenation of events sustains the interpretation. And the conclusion the course is temperature directed.

Rate-temperature-culturetime correlation is not to be expected in inter-activity (init vs. prol), inter-structure (hyd vs. gon), or inter-order (2nd vs. 3rd) comparisons. Accessory endogenous factors of rate are too disparate. And potentially disruptive of the 3-way correlation (1950).

To sum up: Progressive strong rise and subsequent fall as season advances is characteristic of, and virtually specific to, rate of proliferation from sources arising during culture. The course is like that of

temperature. And period-to-period changes in rate tend to be positively correlated with those of temperature. The conclusion is that the course is temperature, which is to say season, determined. Because of colony weakening by culture conditions at time of appearance of activity.

Proliferation and Temperature. Two items other than the foregoing: rate-temperature correlation is more frequently positive in proliferation than initiation. The differential derives from the fact that processes of proliferation differ from those of initiation in kind, number, and complexity (1936). Its direction supports the opinion that the proliferation complex is particularly sensitive to temperature.

Positive correlation is more frequent in hydranths than gonangia. Basic processes are the same. Season factors are the same, but accessory endogenous factors differ in kind, because of an ontogenetically determined difference in chemical composition; in quantity because cell specialization is a greater contaminant in gonangia (1950). This may be major, since the differential is greater in differentiation than proliferation, as will be shown in later report.

Proliferation variability: of each expression by month of all years combined; and by year of all months combined is in Table 3. Computed from Table 46 (1950). Wanted is relative variability by expression; by month; by year. Procedure is in 1951 report. Relative rates similarly gotten from Tables 36; 48 (1950) are also given.

The 6 expressions in hydranths: the 8 in gonangia fall in following order of decreasing variability; increasing rate:

	Hydranth
Variability	CO > SN > AN > EM > BR > Bd
Rate	CO < SN < AN < EM < BR < Bd

Gonangia	
Variability	Bd:3 > AN:3 > MQ:2 > AN:2 > Bd:2 > QT:1 > MQ:1 > Bd:1
Rate	Bd:3 < AN:3 < MQ:2 < AN:2 < Bd:2 < QT:1 < MQ:1 < Bd:1

The seriations are reliable because alike in years and months. Where no progression occurs on one basis (3 cases) such is found on the other (*Hyd*-Var. Month AN=EM; Year AN EM. *Gon*-Var. Year MQ: 1 = Bd: 1; Month MQ: 1 Bd: 1. Rate Year AN: 2 = Bd: 2; Month An: 2 Bd: 2).

Variability by year is season variability. The concordance of this with variability by month shows the gamut is not season induced, because exogenous factors differ in different months. The basis of the seriation is endogenous because the step-by-step decrease in variability exactly matches that of increase in rate in overall; and rate differentials

are endogenously determined (1950). The higher the rate, the closer the approach to expression of potentiality within the 24-hour time-slice. The closer the approach, the greater the stabilization of the subtending processes; and the greater the stabilization the less the variability (1951).

Supportive is the fact that variabilities are higher in gonangia than hydranths (order for order), because rates are lower. And stabilization of processes would be expectedly less in gonangia because their growth depends on hydranth production and feeding (1950). The differential is as in initiation, which is added evidence of direct relation between variability and dependence of a part on the whole: between variability of a part and its importance to the whole (1951). All is consistent.

Variability by month is the same in hydranths and gonangia in order of decrease: viz.—

Variability	AP > MY > JN > SP > AG > JY
Rate	AP < MY < JN < SP < AG < JY

The concordance in the two structures attests the validity of the month-to-month differentials and the seriation. The progressive decrease from April to June is taken as product of advancing maturity. The low of July is presumably due to attainment thereof and the general stabilization resulting therefrom (1950; 1951). The exactness of inverse relation to increase in rate is further evidence that endogenous control is primary.

Variability by year is—in order of decrease:

Hydranths	
Variability	'38 > '39 > '35 > '33 > '36 > '37 > '34
Rate	'38 < '37 < '39 < '35 < '36 < '34 < '33
Gonangia	
Variability	'39 > '37 > '38 > '36 > '35 > '35
Rate	'39 < '38 < '35 < '37 < '36 < '34

Though the seriations differ along the line they coincide at their extremes. 1939 is year of high variability: 1934 of low. As happened in initiation. The high-low differential is opposite that of rate. Endogenous conditioning holds here despite smothering elsewhere. Which is presumably due to cancellations from differences in nature, rates, endogenous accessory factors, exogenous factors in different months, and timing in season shift in such in different years.

This completes the recital of variability, and of rates on season. A possibility deserves note: unwonted cell division is characteristic

of cancer. Heat, sunburn, burns, trauma and irritation which bring localized rise in temperature, are factors in cancer incidence. Proliferation processes seem specially sensitive to temperature. It may be this property of this activity contributes to cancer growth.

Summary and Conclusion

Proliferation has 14 expressions of record in Obelia colonies during a 24-hour time-slice in culture: 6 hydranth, 8 gonangial. The course of rate change over the months from April to September shows two types of trend.

That from sources present at start of culture before such is important has three phases: an initial high due to colony youngness; a low followed by rise due to temperature; and a tapering off to virtual asymptoticism due to colony maturing. Circa 66 per cent of the course is endogenously determined: 34 per cent by temperature. The dichotomy is due to particular sensitivity of proliferation processes to temperature plus heightened reactivity of organism to exogenous influence during time of growingupness.

Proliferation from sources arising during culture starts low, then rises strongly to a peak, to fall away again as season advances. The course is product of season change in temperature, resulting from delay in expression of the activity in culture to a time when colonies are somewhat weakened by culture conditions.

Different expressions differ in variability. This is inversely related to difference in rate. The seriations are endogenously determined, and derive from relative contamination of the activity by accessory endogenous factors. Gonangial proliferation is more variable than hydranth, because dependent on hydranth production and feeding for substance. April is month of greatest variability because of colony youngness, July is month of least, because colony activity-as-a-whole then tends to stabilization.

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